PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF ILEX CRENATA 'ROTUNDIFOLIA' TO SUPRAOPTIMAL ROOT-ZONE TEMPERATURES

By

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Three attributes of the response by Ilex crenata Thunb. 'Rotundifolia' to supraoptimal root-zone temperatures were examined. In the first experiment, split-root plants were grown for three weeks at root-zone temperatures of 30/30, 30/34, 30/38, 30/42, 34/34, 38/38 and 42/42 C to look at the effects of root-zone temperature on ^{14}C-photosynthate partitioning. The 38 C root-zone temperature treatment was the upper threshold for a number of growth and physiological parameters. Higher root-zone temperatures did not affect photosynthetic rates. No differences were found for total ^{14}C partitioned to the roots but partitioning of ^{14}C into soluble and insoluble fractions and the magnitude of root respiration and leakage were influenced by treatment. Heating half the root system at 38 C increased the amount of ^{14}C respired from the heated side and increased the total CO_2 respired from the non-heated (30 C) half. Exposure of both root halves to 42 C resulted in substantial loss of ^{14}C photosynthates through leakage into the media, presumably due to membrane damage.

Respiration rates for excised roots grown at 30, 34, 38 and 42 C decreased linearly with

increased root-zone temperature when the buffer solution was maintained at 25 C. Lack of differences in respiration rate of excised roots when respiration was measured at the same temperature as the root growth temperature was in agreement with respiration results from the split-root plant experiment. A 10 minute exposure to 46 C was sufficient to decrease respiration and reduce the activity of the CN-resistant pathway in 'Rotundifolia' holly. Studies with split-root plants and excised roots indicated that respiratory mechanisms were functional to 42 C.

There was no evidence of stomatal inhibition of CO_2 assimilation due to increased root-zone temperature. No differences in chlorophyll *a* fluorescence were detected, indicating that photosynthetic electron transport was not affected by root-zone temperature. Chlorophyll levels decreased linearly with increased root-zone temperatures. Increases in RuBisCO activity indicated that 'Rotundifolia' holly altered metabolism to maintain photosynthetic assimilation at increased root-zone growth temperatures.

CHAPTER I

REVIEW OF THE LITERATURE

Introduction

Heat stress is of economic importance since it reduces the growth of container-grown plants. The annual sales of woody ornamentals in the state of Florida exceeds $150 million annually. Eighty to eighty-five percent of these plants were container-grown. Plants grown in containers often experience a range of supraoptimal root-zone temperatures during the summer months. This is due to direct and reflected solar radiation on container walls. Absorption of radiation is affected by container color, container design and spacing and orientation of containers (Ingram, 1981; Ingram et al., 1988). Roots are known to be less adaptive and more sensitive to temperature extremes and fluctuations than shoots and, therefore, may not be able to sufficiently adapt physiologically and biochemically to extreme temperature changes.

While considerable work has been conducted to quantify the physiological and biochemical responses of shoots to supraoptimal air temperatures, minimal efforts have been directed towards the influences of supraoptimal root-zone temperatures on plant metabolic processes. Such work is necessary to determine those physiological and biochemical parameters which may limit growth or potentially cause necrosis of herbaceous and woody plants when roots are exposed to supraoptimal temperatures.

The overall goal of this research was to determine the basis for the effects of supraoptimal root-zone temperatures on decreased net photosynthetic rates and to identify factors which regulate carbon distribution and photosynthetic efficiency. Recovered $^{14}CO_2$ photosynthate in

a woody species has been found to decrease with increasing root-zone temperatures (Foster, 1986). Two mechanisms for the decrease in recovered photosynthate were proposed: 1) a decrease in CO_2 fixation, and/or 2) an increase in photosynthate translocation and subsequent evolution through root respiration. The specific objectives of this research were to 1) determine photosynthetic sink strength and carbon loss from roots, 2) to characterize root respiration, and 3) to determine physiological and biochemical photosynthetic responses of plants, all in response to supraoptimal root-zone temperatures.

Photosynthate Partitioning

Carbon Transport

Carbon flow in plants is dependant upon photosynthetic carbon assimilation. Plant growth and yield involves the integration of photosynthetic carbon assimilation in source leaves and its subsequent allocation to and utilization by sink tissues. Source/sink relations may be viewed in terms of the source being equated with provision and the sink with consumption of photosynthates, although more rigid interpretations exist. In C_3 plants, Herold (1980), referred to the source as being restricted to the chloroplast which is the site of photosynthetic carbon fixation. A sink may include any non-photosynthetic plant part which is dependant upon the chloroplast for carbohydrates.

While a number of environmental factors may influence photosynthetic carbon assimilation, demands by sink tissues for assimilate may also determine photosynthetic supply (Gifford and Evans, 1981; Bunce and Ward, 1986). The term 'mobilizing ability' of assimilates was coined by Wareing and Patrick (1975) to define the ability of a particular sink to attract assimilates in the presence of competing sinks. Mor and Halevy (1979) used the term "percent relative specific activity" (%RSA) to quantify the mobilizing ability of a plant system. The %RSA allows for the comparison between different sinks within the same plant as well as comparisons between similar sinks in different plant parts, regardless of tissue weight.

Pulse-chase labelling of plants with $^{14}CO_2$ has been used to study the allocation and accumulation of recently-fixed carbon in different plant parts. Such studies include the partitioning of photosynthates during the reproductive periods of different bean species (Kuo et al., 1978; Geiger and Shieh, 1988), anoxia in roots of common bean (Schumacher and Smucker, 1985), seasonal distribution in ponderosa pine (Smith and Paul, 1988), cold-hardening of citrus (Guy et al., 1981) and heat stress in tomato (Dinar and Rudich, 1985a), among others.

Photoassimilates transported to the roots of plants may be utilized by a variety of carbon sinks (Lambers, 1987). The major sinks include exudation, growth, respiratory processes and symbiotic associations such as nitrogen fixation and mycorrhizae. A number of researchers have successfully used ^{14}C-labelling techniques to study the movement, respiration and exudation of photoassimilated compounds (Martin, 1975; Barber and Martin, 1976; Martin, 1977; Martin and Kemp, 1980; Whipps, 1984; Whipps, 1985; Schumacher and Smucker, 1985; and Douds et al., 1988).

Plants release soluble exudates (amino acids, amides, reducing sugars) and insoluble organic material such as mucilaginous compounds (Lambers, 1987). The loss of carbon by exudation and/or root leakage is generally considered to be 5% or less of the total partitioned photosynthate, though this may vary under adverse conditions. The extent to which organic compounds are released from plant roots into the rhizosphere was dependant upon the physical and chemical properties of the medium in which the plant is being grown (Martin, 1975). The release of carbon was found to be dependant upon the degree of localized water stress on the roots. Dinwoodie and Juma (1988) found differences in soil ^{14}C between two soils cropped to barley to be due to variations in water filled porosity. Soluble carbon, used as a measure of exuded carbon, increased in soil with more than 70% water filled porosity. Merckx et al. (1985) found carbon turnover rates in sandy soils to be greater than rates in clay soils.

Environmental Factors

A variety of environmental factors such as temperature, daylength and anaerobiosis influence the loss of carbon compounds from roots (Whipps, 1984). Martin (1971) studied the loss of ^{14}C water soluble material leached from the rhizosphere of wheat, ryegrass and clover. After six days the bulk of the radioactivity was recovered from the soil microflora. Organic compounds from root exudation and lysis were the main source of substrate for soil microflora. Martin (1977) found the presence of microorganisms increased the release of $^{14}CO_2$ from the rhizosphere, but had no effect on the ^{14}C content of the soil. He determined that the major loss of root carbon from wheat during the 3- to 8-week study period was due to the autolysis of the root cortex. Roots of different cereal crops in sterile media released 5 to 10% of the photoassimilated carbon compared to 12 to 18% by roots of plants being grown in unsterilized media (Barber and Martin, 1976).

Irradiation has been used as a technique for sterilizing media. Whipps and Lynch (1983) found the CO_2 derived from respiration of roots and microorganisms to constitute the largest fraction of the ^{14}C released from the roots (15 to 20% of the total) and this was irrespective of irradiation treatment. Carbon loss through root exudation from three grass species, Agropyron cristatum, A. smithii, and Bouteloua gracilis, was estimated to be 8, 17 and 15%, respectively, of the total C fixed (Biondi et al., 1988). Root exudation for A. cristatum and A. smithii significantly increased in the presence of rhizosphere microorganisms. The amount of C released under sterile conditions compared to nonsterile conditions was 17 and 60% for A. smithii and A. cristatum, respectively. No difference was found between sterile and nonsterile grown B. gracilis in terms of root exudation thereby indicating this species may be inherently insensitive to the presence of soil microflora.

Total carbon loss from plant roots through microbial and root respiration (which are not directly separable) and carbon remaining in non-sterile soils can be estimated using $^{14}CO_2$-labelling techniques (Whipps and Lynch, 1984). The use of sand as a plant growth medium has

been a useful technique for evaluating the movement of ^{14}C compounds into media (Bennett and Lynch, 1981; Whipps and Lynch, 1983; Douds et al., 1988).

Temperature also influences the loss of carbon from root tissue. Martin (1977) grew wheat at 10 C and 18 C and found the ^{14}C loss from the roots to be 14.3 and 22.6% of the total transported to the roots and the ^{14}C translocated to the roots to be 29.2 and 44.4% of the total net fixed, respectively. Martin and Kemp (1980) determined that a reduction in growth temperature for wheat in the range of 10 to 15 C increased carbon loss from the roots into the rhizosphere. They noted that since the roots and shoots were grown at the same temperature it could not be determined if the effect of temperature on carbon loss from the roots was due to differences in shoot temperature, root temperature or an interaction between the two.

Translocation to and loss of carbon from roots varies greatly among species. Using wheat grown in the field, it was determined that when a single pulse of $^{14}CO_2$ was used, approximately 27% of the total fixed CO_2 was lost as respiration from roots and soil (Martin and Puckridge, 1982). Whipps (1984) grew wheat for 21 days and noted a loss of 34 to 40% of net fixed carbon or a loss of 67 to 68% of the ^{14} C translocated to the roots. For corn grown at different CO_2 concentrations for periods of 14 and 28 days, Whipps (1985) concluded that 47 to 69% of the ^{14}C translocated to the roots was lost through root leakage and respiration. It was determined that most of the $^{14}CO_2$ assimilated by corn plants was translocated within the aboveground portion of the plant (approximately 90%) (Davenport and Thomas, 1988). In bromegrass, the amount remaining in the aboveground portion was approximately 60%. After one year of cropping, the amount of rhizodeposition for bromegrass was twice that of corn in the top 15 cm of soil. While no difference was detected for root exudate and media ^{14}C, root ^{14}C was approximately five times higher for bromegrass compared to corn. Lambers (1987) estimated that up to 30% of the photoassimilated carbon translocated to the roots is lost through respiratory processes. This value included growth and maintenance respiratory portions as well as contributions from the cyanide-resistant, alternative respiratory pathway.

Symbiotic Associations

Nitrogen fixation and mycorrhizal symbiosis have been estimated to consume 5 to 23% and 7 to 10%, respectively, of the total daily photoassimilated carbon transported to the roots (Lambers, 1987). Nitrogen nutrition is considered to be an integral part of the carbon economy of nodulating,leguminous plants. Considerable research has been conducted on soybeans using $^{13}CO_2$ tracer techniques to study the movement and respiration of photoassimilates in nodulated plants (Kouchi and Yoneyama, 1984a; Kouchi and Yoneyama, 1984b; Kouchi et al., 1985; Kouchi et al., 1986a; Kouchi et al., 1986b). Kouchi et al. (1985) demonstrated that currently assimilated carbon was preferentially used to support nodule respiration while root respiration was dependant upon earlier non-labelled carbon reserves located in the roots. Starch was not considered to be an important part of this reserve pool for root respiration.

Kouchi et al. (1986a) determined that the total amount of labelled carbon transported into the nodules accounted for up to 35% of the total photoassimilate. Labelled CO_2 respired by the roots was significantly less than that respired by the nodules. It was again noted that the roots contained reserve materials which supported root respiration. When the supply of photoassimilates to the nodules was limited, the reserve materials supporting root respiration were not remobilized to aid nodule respiration. Starch synthesized during the light period was found to be preferentially degredated and mobilized to the root system during the dark period to support respiration.

Kouchi et al. (1986b) used a compartmental analysis model to study the partitioning of photoassimilated carbon in nodulated soybean plants. Their analysis showed: 1) the largest carbon flux during the light period was to storage materials in above-ground organs, 2) the main carbon flux to the nodules was via direct phloem movement from the shoot and not via root soluble compartments, 3) a large portion of the carbon transported to the roots was returned to the shoots and 4) the root soluble carbon pool acted differently from soluble pools in other organs. The labelled carbon component in the soluble root pool increased continuously

over the entire experimental period. This suggested that the root soluble pool continually replenishes carbon which acts as a reserve respiratory pool. This is in agreement with previous research (Kouchi et al., 1985; Kouchi et al., 1986a).

Mycorrhizae depend upon photoassimilates from the plant and, therefore, may serve as a sink for carbon compounds. The degree of exudation from roots with mycorrhizal associations correlated positively with the degree of infection (Graham et al., 1981; Johnson et al., 1982). Much of the ^{14}C-labelling work with mycorrhizal fungi has been done in conjunction with studies regarding phosphorous nutrition (Snellgrove et al., 1982; Koch and Johnson, 1984; Douds et al., 1988). Snellgrove et al. (1982) found approximately 7% more total fixed carbon being translocated to the roots of mycorrhizal leek plants compared to non-mycorrhizal plants. The extra photoassimilate translocated to the roots could be accounted for via increased root respiration and increased loss of carbon into the medium. Both mycorrhizal and non-mycorrhizal plants had equal rates of carbon assimilation per unit leaf area.

Koch and Johnson (1984) used split-root sour orange and Carrizo citrange plants to study the effects of photoassimilate partitioning to mycorrhizal and non-mycorrhizal root halves. The mycorrhizal root-half for the sour orange and Carrizo citrange accumulated 1.91 and 1.96 times, respectively, more labelled photoassimilate compared to the non-mycorrhizal half, indicating the mycorrhizae serve as a preferential sink for current photoassimilate. They concluded for plants with a root system which were totally mycorrhizal the estimated cost of current photosynthate for the sour orange and Carrizo citrange would be 6.0 and 10.6%, respectively. Douds et al. (1988) using split-root plants of Carrizo citrange which were mycorrhizal, half mycorrhizal, or non-mycorrhizal determined that 3 to 4% of the current photosynthate was allocated towards the metabolism of mycorrhizae independent of treatment. Labelled compounds found in the medium were 5 to 6 times greater for mycorrhizal roots compared to non-mycorrhizal roots. Non-mycorrhizal roots were found to respire more currently-labelled photoassimilate than mycorrhizal roots. This may be a characteristic of the mycorrhizal association or due to the

short labelling period during the study (2 hours). In contrast, mycorrhizal roots of soybean (Harris et al., 1985) and leek (Snellgrove et al., 1982) displayed increased respiration rates compared to non-mycorrhizal roots, though this may vary with the age of infection (Harris et al., 1985). Smith and Paul (1988) working with ponderosa pine demonstrated that mycorrhizal roots during certain periods of the year became a large sink for carbon compared to nonmycorrhizal roots. Their research indicates that 30 to 40% of the carbon assimilated remains in the tree with the remainder being respired.

High temperatures influence assimilate partitioning. Much work has been conducted to understand the influence of high temperature stress on carbon assimilation and export rates in tomatoes (El Ahmadi and Stevens, 1979; Dinar et al., 1983; Bar-Tsur et al., 1985; Dinar and Rudich, 1985a; Dinar and Rudich, 1985b). El Ahmadi and Stevens (1979) studied the effects of high temperatures on assimilate partitioning in several heat-tolerant tomato cultivars. In all of the cultivars except one, flower production decreased due to high temperature (35 C) inhibition of the partitioning process. Assimilate export in tomato leaves was dependant upon assimilation rate of leaves, while the rate of import into a sink organ was influenced by the utilization rate of the organ (Ho, 1979).

Using tomatoes it was determined that at increased temperatures, basipetal transport to the roots and assimilate transport to young flowers was more pronounced in Roma VF (heat-sensitive) than in Saladette (heat-tolerant) (Dinar and Rudich, 1985a). Upward movement of assimilate decreased in response to heat stress. The authors concluded that heat stress appears to affect sink strength or the ability of young flower buds to attract assimilates. Dinar and Rudich (1985b) also found differences in sucrose and starch levels under heat stress conditions. As temperature increased, the levels of sucrose in the source organ increased while the levels of starch decreased. Transport of $^{14}CO_2$ was correlated with the starch content of the flower buds. This conversion of carbon into starch was more prevalent for the heat-tolerant cultivar, Saladette, compared to the heat-sensitive cultivar Roma VF.

Increased root-zone temperatures can also have profound effects on growth, assimilate partitioning and respiratory mechanisms of plants (Cooper, 1973; Nielsen, 1974). For curled parsley, root-zone temperatures of 36 C severely inhibited root and shoot growth (Eidsten and Gislerod, 1986). Short exposures of root-zone temperatures above 30 C were shown to retard growth. Shishido et al. (1987) found ^{14}C exported from the source leaf of cucumber to the root increased as leaf temperature increased from 15 to 30 C. Up to 45% of the exported ^{14}C was lost via respiration. Maletta and Janes (1987) determined that continuous root-zone temperatures of 32 C were detrimental to the growth of tomato seedlings. Shoot and root growth, root carbohydrate levels and photosynthetic rate decreased in Pittosporum tobira over a seven month period in which plants were exposed to a root-zone temperature of 40 C for six hours per day (Johnson and Ingram, 1984). In one study, Foster (1986) found mean respiration rates for roots of 'Rotundifolia' holly to increase with increasing root-zone temperatures from 28 to 40 C. He also found that increasing the root-zone temperature decreased the percentage of recovered ^{14}C - photosynthate in the roots. The reasons for the decrease in recovered photosynthate were proposed to be due to 1) decreased translocation to the roots, 2) increased carbon loss due to increased root respiration, and/or 3) decreased CO_2 fixation.

Respiration

Growth and Maintenance

Respiration is a process in which substrates are oxidized to release energy in living cells. This energy is stored as chemical energy in substrate molecules and can be used to maintain protoplasm structure (maintenance respiration) or in synthesis processes (growth respiration) (Kramer and Kozlowski, 1979). Penning de Vries (1975) has described maintenance respiration as CO_2 resulting from protein breakdown and CO_2 which is produced in respiratory processes to provide energy for maintenance processes. Maintenance processes may be those involved in maintaining cellular structure and ion gradients as well as processes involved in the

physiological adaptations necessary to maintain cell function in a changing environment. Maintenance processes considered to occur under normal growing conditions include the breakdown and resynthesis of nitrogenous compounds and lipids. These are functions of the turnover process necessary for plants to survive in their environment. Growth respiration is the portion of respiratory substrate used for the production of dry matter (Penning de Vries, 1974).

Plant tissue will often show a marked increase in respiratory rates up to temperatures of 40 to 45 C. Increases in temperature can have profound effects on maintenance costs in plant cells due to increased ion fluxes and protein turnover (Penning de Vries, 1975). Therefore, maintenance respiration is strongly temperature dependant since it has been shown to be directly related to enzymatic degradation processes (Johnson and Thornley, 1985). Dark and maintenance respiration have been shown to have a Q_{10} (respiration rate at T + 10 C divided by the respiration rate at T, where T = temperature) of 2 or more in a variety of plants (Penning de Vries, 1974; McCree, 1974; Hunt and Loomis, 1979). Kase and Catsky (1984), using C_3 (bean) and C_4 (corn) plants, found dark respiration and maintenance respiration to increase with temperature in the range of 15 to 40 C for bean and 15 to 62 C for corn. Growth respiration was temperature dependant with a optimum temperature similar to that of the optimum for gross photosynthesis.

Increased root temperatures have been shown to increase the maintenance portion of root respiration in sunflower, and to a lesser degree, shoot maintenance respiration (Szaniawski and Kielkiewicz, 1982). Szaniawski (1981) found maintenance and growth respiration to be greater in the roots than in the shoots of Scots pine seedlings. However, Ledig et al. (1976) found maintenance and growth respiration to be substantially higher for shoots compared to roots in seedlings of pitch pine. Supraoptimal temperatures have been shown to increase maintenance respiration while causing decreases in growth due to decreases in the available carbohydrate pool (Gent and Enoch, 1983).

Root respiration is an important component in the carbon economy of a plant. Gent and Enoch (1983) developed a model to describe the influence of temperature on the carbon metabolism and growth of plants. The model suggested that growth of carnation and tomato was limited by a shortage of respiratory energy at low temperatures and was limited by a shortage of carbohydrates required at high temperatures. This is consistent with high temperature induced starvation because the depletion of plant reserves is an important factor in decreased plant growth and yield (Chen et al., 1982).

Alternative Respiratory Pathway

Considerable research has been conducted in recent years on the different respiratory pathways associated with plant mitochondria. While respiration in animals in known to be extremely sensitive to inhibitors such as cyanide (CN), a separate component can be found in most plant tissues which is insensitive to CN^-, CO and N_3^- (Douce, 1985). In plants there are two known mitochondrial electron transport terminal oxidases, the first being cytochrome oxidase which is CN-sensitive, and the less understood alternative oxidase which is CN-resistant (Solomos, 1980). The CN-insensitive or resistant pathway in plant mitochondria is considered to be an alternative, non-phosphorylating pathway which branches from the respiratory chain at ubiquinone (Henry and Nyns, 1975). While the CN-resistant pathway itself is non-phosphorylating, oxidation of TCA cycle intermediates can lead to the synthesis of a limited quantity of ATP (Siedow and Berthold, 1986). Due to the branching of the CN-resistant pathway at ubiquinone, two sites of energy conservation are by-passed (complexes III and IV). The maximal P/O ratio (moles of inorganic phosphate recovered in organic form per atom oxygen consumed) possible from electrons derived from the TCA cycle will be 1. This can occur because of the oxidation of NADH at complex I, located between the mitochondrial matrix and the inner membrane. A P/O ratio of 0 is also possible since exogenous NADH or succinate from the matrix may also be oxidized. The P/O ratio of 0 indicates there is no phosphorylation site between ubiquinone and the alternative oxidase.

Physiological Role of the CN-resistant pathway

The physiological role of the CN-resistant pathway as well as its regulation is still uncertain. The degree of engagement of the CN-resistant pathway is quite variable and except for certain of the Araceae, where thermogenesis is know to occur, the role of the pathway is unclear. Two models have been proposed for the regulation of electron transport through the cytochrome and CN-resistant pathways. The CN-resistant pathway became engaged in mitochondria of isolated mung bean hypocotyls when the CN-sensitive pathway became saturated (Bahr and Bonner, 1973). This occurred when the concentration of reduced ubiquinone was high enough to allow for the thermodynamically favorable oxidation of ubiquinol by the alternative oxidase. Thus, the basis of this model was that electrons will "spill-over" into the CN-resistant pathway only when the CN-sensitive pathway was saturated. The second model suggested by de Ttroomstembergh and Nyns (1978) was that electron flow was partitioned between both pathways according to the relative rate constants of the reactions between ubiquinol and the cytochrome and alternative paths.

The one known physiological role of the CN-resistant pathway is thermogenicity, or the production of heat in the floral structures of certain families of plants (Robacker et al., 1988). This rapid generation of heat through induction of the alternative path is important for the generation of floral aromas which attract pollinators. The endogenous agent which triggers heat production in the flowers and inflorescences of thermogenic plants has been called "calorigen" over the years. Recently, Raskin et al. (1987) found salicylic acid to be the endogenous regulator of heat production in Arum lilies.

Lambers (1982) suggested that the CN-resistant pathway functions as an energy overflow which removes excess carbohydrates when the substrate level in the cell is high and the need for carbon skeletons and ATP is low. Day and Lambers (1983) demonstrated that glycolysis could be regulated in roots of several species which show CN-resistant pathway activity. Regulation occurred through equilibrium displacement of phosphofructokinase and pyruvate

kinase reactions. Lambers (1983a) provided supporting evidence for his overflow hypothesis by citing a number of correlations between respiration rate, sugar content and the engagement of the CN-resistant pathway in a number of tissues. Using leaves of wheat and spinach, Azcon-Bieto et al. (1983) demonstrated that the CN-resistant pathway was not engaged in the morning when carbohydrate levels were low, but became engaged when carbohydrate levels increased after several hours of photosynthetic activity. Blacquiere et al. (1987) found elevated root respiration rates and CN-resistant pathway activity to be associated with decreased soluble sugar levels in the roots of two Plantago species. This was not consistent with the energy overflow model. Oaks (1986) found the alternative oxidase in corn roots to be less active in the presence of glucose than in its absence. The author concluded that the contribution of the CN-resistant pathway to oxygen uptake was related to the metabolic demand of cells, but was not actually related to carbohydrate levels or supply.

Day et al. (1985), using two different populations of ryegrass, found leaf respiratory rates were limited by adenylate control of glycolysis. Differences in leaf respiration rates between populations were unrelated to the engagement or presence of the CN-resistant pathway. Under circumstances where a sudden increase of cellular ATP was required, the CN-resistant pathway may be engaged to provide an inefficient mechanism for producing ATP. This concept is known as the "overcharge overflow" model (de Visser et al., 1986).

Alternative Oxidase

The biochemical nature of the alternative oxidase has not been thoroughly elucidated at this time. Bonner and Rich (1978) have suggested that the alternative oxidase is a ubiquinol:O_2 oxidoreductase. Rich (1978) and Huq and Palmer (1978) showed that a salicylhydroxamic acid (SHAM)-sensitive quinol oxidase could be solubilized from aroid spadix mitochondria. An iron-containing aroid oxidase with an apparent molecular weight of 33 kD has recently been described (Bonner et al., 1984). The alternative oxidase was believed to be an iron-containing protein which was neither a haemoprotein or iron-sulphur protein. Supportive evidence for this

theory included: 1) the oxidase was inhibited by hydroxamates known to chelate transition metals, 2) the inhibition was counteracted by Fe^{2+} ions, and 3) the iron in iron-sulphur proteins was not chelated by hydroxamates (Goodwin and Mercer, 1983). This concept is no longer generally accepted since not all of the mitochondrial iron-sulphur proteins suspected of being the alternative oxidase exhibit hydroxamate inhibition (Douce, 1985).

Various methods including electron paramagnetic resonance have been used in an attempt to identify the alternative oxidase, yet no spectroscopically identifiable species have been found (Siedow and Berthold, 1986). Berthold et al. (1987) have used the radiation inactivation analysis technique to estimate a functional molecular weight for the alternative oxidase as being 38,000. This method is based on the premise that an enzyme with a larger mass will intercept more ionizing radiation and will, therefore, be inactivated due to the large dosages intercepted. The authors suggested the functional molecular weight of 38 kD corresponds to a single polypeptide, but could not rule out the possibility of several proteins existing of which 38 kD was the largest component.

Elthon and McIntosh (1987) using nickel stained SDS-PAGE procedures have purified a cluster of proteins near 35 kD from the alternative oxidase of Sauromatum guttatum floral appendix mitochondria. With their partially purified alternative oxidase, they determined that lipids were required for optimal activity. Loss of activity resulted from the loss of essential lipids from the alternative oxidase protein(s). Phospholipids were found to be the most effective in restoring functional activity of the oxidase. Polyclonal antibodies from mice were also effective in precipitating the protein(s) responsible for the alternative oxidase activity. Walsh and Moore (1987) used immunological techniques to study the alternative oxidase. Antisera raised against partially purified alternative oxidase from solubilized Arum sub-mitochondrial particles was capable of immunoprecipitating SHAM-sensitive and antimycin A- insensitive quinol oxidation, therefore suggesting that this activity was associated with a protein.

Rustin (1987) reviewed the current hypothesis regarding the terminal oxidation step of the alternative respiratory pathway. The four current hypotheses include: 1) quinol auto-oxidation, 2) lipoxygenase, 3) quinol oxidase and 4) free radical mechanisms. Quinol auto-oxidation has been proposed since ubiquinone is the electron carrier generally considered to be involved with the CN-resistant pathway and the fact that its auto-oxidation is SHAM-sensitive. The two major points against this mechanism are that 1) quinol auto-oxidation does not account for the appropriate stoichiometry between substrate and oxygen and 2) quinol auto-oxidation is sensitive to superoxide dismutase whereas other CN-resistant oxidations are not, therefore its response to inhibitors is not similar.

Lipoxygenase has been implicated as being responsible for CN-resistant respiration since it is CN-resistant yet is sensitive to SHAM and n-propylgallate like the alternative respiratory pathway. However, it is now generally accepted that lipoxygenase is a contaminating enzyme in mitochondrial preparations and is not the terminal alternative oxidase. Scherban and McDaniel (1987) found that CN-insensitive oxygen uptake by mitochondria from soybean cotyledons was associated with lipoxygenase activity which can be removed using Percoll gradients. Lipoxygenase catalyzes oxygen uptake by oxidizing linoleic acid and may interfere with measurements of the alternative respiratory pathway. Day et al. (1988) concluded that the alternative oxidase activity in soybean cotyledons was a constitutive property of the respiratory chain and not the result of contaminating lipoxygenase. Quinol oxidase has been considered a candidate for the terminal alternative oxidase due to its CN-resistance, SHAM and propylgallate-sensitive quinol oxidation in mitochondria from some members of the Araceae (Rustin, 1987; Elthon and McIntosh, 1987). Rustin (1987) reported that little similarity existed between quinol oxidase activity from sub-mitochondrial particles of Arum maculatum and the system responsible for CN-resistant terminal oxidation. Quinol oxidase activity has only been solubilized from mitochondrial fractions of plants in the Araceae and this activity has not been found in other plants.

The final hypothesis which Rustin (1987) proposed was a free radical mechanism. According to this hypothesis, ubiquinol (QH_2) can be reoxidized in the presence of free unsaturated fatty acid peroxyl radicals (ROO*) which in the mitochondrial membranes act as electron acceptors (Rustin et al., 1983; Douce, 1985). Rustin et al. (1983) suggested that reduced quinones (duroquinol) and fatty acid peroxyl radicals (ROO*) interact in such a manner as to be CN-insensitive yet sensitive to inhibitors of the CN-resistant pathway. The proposed reaction product of ROO* is ROH which upon reoxidation yields H_2O, not H_2O_2, as a terminal product. This is in line with Siedow (1982) who determined the product of reduction of O_2 by the CN-resistant pathway was H_2O and not superoxide or hydrogen peroxide. Rustin (1987) offers three lines of evidence in support of the free radical mechanism and concludes that this mechanism could account for all the known features of the CN-resistant terminal oxidation steps. The hypothetical scheme for the free radical mechanism first involves the production of radicals (R*) and peroxy radicals (ROO*) of linoleic acid. The peroxy radicals of linoleic acid react with two duroquinol molecules which leads to the oxidation of duroquinol to water with the regeneration of a free radical.

CN-resistant pathway Inhibitors

The alternative respiratory pathway is insensitive to the inhibitor antimycin A but is inhibited by substituted hydroxamic acids such as salicylhydroxamic acid (SHAM) (Schonbaum et al., 1971). This inhibition by hydroxamic acids varies with the type of hydroxamic acid used and the source of isolated plant mitochondria. The antioxidant n-propyl gallate was shown to inhibit the CN-resistant pathway (Siedow and Girvin, 1980). Kinetic studies indicate that propyl gallate and hydroxamic acids inhibit the pathway at the same site, probably because of the geometric similarity between the two molecules. Siedow and Bickett (1981) determined that the dihydroxyl function of propyl gallate was responsible for inhibition. Siedow and Bickett (1983) were later able to use a propyl gallate analog, butyl gallate, to estimate the amount of alternative oxidase present in mung bean mitochondria. Other compounds such as adenine derivatives

(Dizengremel et al., 1982), cytokinins (Miller, 1979) and disulfiram (Grover and Laties, 1981) have also inhibited the CN-resistant pathway, though not at the same sites as hydroxamates and gallates. Since hydroxamates can be used to inhibit plant mitochondria at low concentrations which have no discernable effects on the CN-sensitive pathway yet completely inhibit the CN-resistant pathway, they have become an important tool for studying the flow of electrons through both pathways (Laties, 1982).

While the use of hydroxamic acids as specific inhibitors in isolated plant mitochondria are widely accepted for determining the activity of the alternative respiratory pathway, interpreting such experiments using intact tissues raises some questions. Concentrations of SHAM up to 25 mM have been used to inhibit the alternative path in intact tissues (Lambers et al., 1983) compared to concentration of 5mM or less for isolated mitochondria. Non-specific inhibition of the CN-sensitive pathway has been seen in isolated mitochondria when high concentrations of SHAM have been used (Schonbaum et al., 1971; Laties, 1982). Although these methods have been questioned, it has been concluded that high concentrations of SHAM provides a reliable estimate of the alternative respiratory pathway activity in vivo and that high concentrations insure complete penetration of the inhibitor into tissue (Lambers et al., 1983a).

SHAM must be used with caution since it was shown to stimulate oxygen uptake in roots of pea (de Visser and Blacquiere, 1984), corn plants (Spreen Brouwer et al., 1986), leaves of soybean (Sesay et al., 1986), soybean cotyledons (Sesay et al., 1988), potato tuber callus (van der Plas et al., 1987) and plasmalemma vesicles from wheat roots (Moller and Berczi, 1985; Moller and Berczi, 1986). The stimulation of oxygen uptake by SHAM was believed to be due to a CN-sensitive peroxidase (de Visser and Blacquiere, 1984; Spreen-Brouwer et al., 1986; van der Plas et al., 1987) or a plasmalemma bound redox chain (Moller and Berczi, 1985; Moller and Berczi, 1986). The oxygen uptake stimulation in roots was thought to be due to an externally located peroxidase which was stimulated by low concentrations of SHAM and inhibited by KCN and high concentrations of SHAM (Spreen Brouwer et al., 1986). van der Plas et al. (1987)

found exogenous NADH to be required for peroxidase activity in potato tuber callus and was not involved in the normal function of oxygen uptake. At least part of the peroxidase activity was located outside of the plasmalemma barrier and could be easily washed from the tissue. Bingham and Farrar (1987) found a SHAM-stimulated peroxidase which could easily be leached from the roots of barley. Oxygen uptake was inhibited by catalase, suggesting H_2O_2 as the end product of the oxidase-mediated reaction. This indicates that the oxidase was a peroxidase. Non-linearity of inhibitor relationships could not be attributed to the SHAM-stimulation of this peroxidase since it was not activated by exogenous NADH. Gentisic acid, a scavenger of superoxide free radicals, was found to suppress much of the SHAM-stimulated activity in detached roots in the presence of NADH but had no effect on SHAM-treated roots without NADH. This suggests that although a peroxidase was present, there was little activity in the absence of exogenous substrate.

For isolated plant mitochondria, the equation for describing total respiration is

$$V_T = p^*g(i)_{alt} + V_{cyt}$$

where V_T is the total respiration rate, V_{cyt} is the cytochrome-mediated respiration and g(i) is the maximal contribution of the CN-resistant pathway at a given concentration of inhibitor (Bahr and Bonner, 1973). The fraction of the CN-resistant pathway which is functioning is defined as p, a number between 0 and 1. Therefore, p*g(i) is equal to the contribution of the CN-resistant pathway. Plant tissue has a residual respiration component (V_{res}) not found in mitochondrial preparations (Theologis and Laties, 1978). Residual respiration is that portion of V_T which is insensitive to inhibitors of the cytochrome and CN-resistant pathways and is considered to be extra-mitochondrial in nature. The origin of residual respiration is not well understood (Lambers, 1987). Thus, the equation for respiration of a plant tissue is defined as

$$V_T = p^*g(i)_{alt} + V_{cyt} + V_{res}$$

The activity of the CN-resistant pathway may be determined by titrating tissue in the presence and absence of SHAM (Bahr and Bonner, 1973; Theologis and Laties, 1978). The

capacity of the CN-resistant pathway (V_{alt}) is the CN-resistant respiration minus V_{res}. The degree of engagement of the alternative path can be calculated from pV_{alt} plots described by Bahr and Bonner (1973) and Theologis and Laties (1978). A necessary assumption for these calculations is that SHAM is specific for the alternative oxidase and does not interfere with other respiratory processes (Bahr and Bonner, 1973; Theologis and Laties, 1978). SHAM may also effect the CN-sensitive pathway and therefore respiration can be titrated with KCN in the presence and absence of SHAM to test for such effects (Theologis and Laties, 1978; de Visser and Blacquiere, 1984). Non-linearity of pV_{alt} plots has been ascribed to the activation of a CN-sensitive oxidase by SHAM (de Visser and Blacquiere, 1984). Bingham and Farrar (1987) concluded that for barley roots, the only factor causing non-linear pV_{alt} plots was the non-specific inhibition of the CN-sensitive pathway by high concentrations of SHAM.

Lambers and Day (1987) suggested the following experimental strategy which allows for the reliable estimation of electron transport in vivo: 1) oxygen electrodes should be used rather than manometers and the use of inhibitors should be restricted to no more than 20 minutes, 2) adequate inhibitor concentrations should be chosen. Respiration should be titrated with KCN and a concentration chosen which maximally inhibits oxygen uptake. Next, respiration should be titrated with SHAM in the presence of KCN and the minimal concentration which maximally inhibits should be chosen, 3) the chosen concentration of SHAM should be applied to the tissue in the absence of KCN to test for the stimulation of oxygen uptake by SHAM. SHAM is believed to stimulate externally located peroxidases at low concentrations (Spreen Brouwer et al., 1986). If oxygen uptake is stimulated by low concentrations of SHAM, it may be necessary to use a higher concentration. At low concentrations of SHAM, oxygen uptake may be stimulated while the CN-resistant pathway is also inhibited. The net effect is no oxygen uptake and this leads to non-linear pV_{alt} plots, and 4) it is important to make sure high concentrations of SHAM do not effect the CN-sensitive pathway. This can be done by reverse titration of KCN in different concentrations in the presence and absence of SHAM. If inhibition

by KCN is the same with and without SHAM, then it can be concluded that no SHAM inhibition of the CN-sensitive pathway occurred.

Environmental Factors

A number of environmental factors such as oxygen concentration, nitrogen nutrition and temperature are known to influence the flow of electrons through the alternative respiratory pathway. Under flooded conditions, the degree of SHAM sensitivity increased and the CN-sensitive pathway activity decreased in the roots of Japanese yew and sugar maple (Carpenter and Mitchell, 1980). The results of their study indicate that both flood-tolerant and flood-intolerant tree species possess CN-resistant and CN-sensitive oxygen consumption mechanisms. Residual respiration was not substantially different in the different species used or as a function of flooding. This indicates that potential cellular oxidases resistant to KCN and SHAM do not account for flood tolerance. It was concluded that continuous flooding causes damage to the aerobic respiratory mechanism of flood-intolerant species, possibly due to the breakdown of cellular oxidases.

The alternative oxidase is known to have a lower affinity for O_2 compared to cytochrome oxidase (Lambers and Smackman, 1978; Solomos, 1980). The CN-resistant pathway has also been shown to be limited by low O_2 concentrations (Lambers and Smackman, 1978; Purvis, 1988). Purvis (1988) suggested that the low solubility of O_2 and/or its slow diffusion rate into tissue together with the low affinity of the alternative oxidase for O_2 may restrict electron flow through the CN-resistant pathway. The results of his study using grapefruit flavedo tissue indicated that the CN-resistant pathway may not be engaged or was not measurable in experimental systems where the tissue was suspended in buffer due to low O_2 concentrations.

As temperature increases, the solubility of O_2 in aqueous solution decreases. McCaig and Hill (1977) suggested this idea as a possible reason for the decline of CN-insensitive respiration of wheat mitochondria above 17.5 C. Therefore, decreased O_2 availability at high temperatures due to decreased O_2 solubility may explain why the alternative path accounts for a greater

portion of total respiration at low temperatures compared to higher temperatures (Cook and Cammack, 1985; McCaig and Hill, 1977; Purvis, 1985; Purvis, 1988).

Respiration in cultured roots of tomato to be highest between 40 and 45 C following a 30 minute incubation period at those temperatures (Janes et al., 1988). This indicated that the enzymes responsible for respiratory processes could perform above those temperatures optimal for growth. The rate of O_2 uptake in leaves of Fatsia japonica in the absence of inhibitors (V_T) increased with increasing temperature up to 25 C with a significant decrease at higher temperatures up to 40 C (Burgos et al., 1987). The capacity of the CN-resistant pathway was high between the temperatures of 6 to 30 C and declined significantly at higher temperatures. The authors concluded that the expression of the CN-resistant pathway in F. japonica leaves at different temperatures was not correlated with CN-resistant pathway capacity but seemed to be dependant upon energy conditions within the leaf.

Temperature has been found to differentially affect the electron transport pathways of CN-resistant and CN-sensitive plant mitochondria (Chauveau et al., 1978). Succinate oxidation in CN-sensitive mitochondria from cauliflower or fresh potato slices was found to disappear after 60 minutes at 40 C. In Sauromatum and Arum mitochondria, as well as aged potato tuber mitochondria, only the CN-resistant pathway was impaired under similar conditions with the CN-sensitive pathway showing resistance to temperature. Oxidative phosphorylation was rapidly destroyed by high temperature treatments in CN-sensitive and CN-resistant mitochondria (Dizengremel and Chauveau, 1978). This response varies between species and under different physiological conditions. In CN-sensitive mitochondria, exposure to 40 C stops all electron transport, whereas only the CN-resistant pathway was impaired in CN-resistant mitochondria. In CN-sensitive mitochondria, the dehydrogenases at the beginning of the electron transport system were particularly sensitive to temperature, thus explaining the decrease seen in electron transport. In CN-resistant mitochondria, the CN-resistant pathway was damaged while the dehydrogenases showed a strong resistance towards thermal inactivation. Therefore, the main

difference between both types of mitochondria was at the level of their dehydrogenases. The authors suggested that a search for changes in polypeptide patterns for inner and outer mitochondrial membranes could yield significant information. Kaderbhai et al. (1987) demonstrated that castor bean mitochondrial protein synthesis had a different response to heat-shock despite the fact that the energy status and respiratory function of isolated mitochondria were unaffected by similar temperature treatments. Protein synthesis increased at temperatures of 12 and 20 C but progressively declined at 28 and 35 C. Isolated mitochondria demonstrated a sharp protein synthesis optimum at 20 C which was different from the optimal physiological growth temperature for this plant (30 C).

Chilling temperatures increased the capacity of the CN-resistant pathway and decreased cytochrome-mediated respiration in soybean cotyledons (Leopold and Musgrave, 1979) and cucumber hypocotyls (Kiener and Bramlage, 1981). Increased CN-resistant pathway capacity was found in a number of plant species at low temperatures (Cole et al., 1982; Cook and Cammack, 1985; McCaig and Hill, 1977; Purvis, 1985; Purvis, 1988; Rychter and Ciesla, 1987; van de Venter, 1985). This indicated that KCN was less inhibitory to the CN-resistant pathway at low temperatures than at higher temperatures (Cole et al., 1982; Cook and Cammack, 1985; McCaig and Hill, 1977; Purvis, 1985). Rychter and Ciesla (1987) found increased respiration rates in winter rape plants at 5 C compared to 20 C were due solely to increases in the CN-resistant pathway. Stimulation of the CN-resistant pathway was accompanied by higher sugar levels resulting from metabolic changes induced by the cold treatment, supporting the hypothesis that sugar levels control the participation of the CN-resistant pathway in leaves (Azcon-Bieto et al., 1983). In tomato seeds, O_2 consumption by the cytochrome and CN-resistant pathways was not affected by different storage temperatures (Beers and Pill, 1986). Kiener and Bramlage (1981) suggested that chilling temperatures in cucumber hypocotyls rapidly increased CN-resistant pathway capacity with a corresponding decrease in the CN-sensitive pathway. They propose that the CN-resistant pathway may serve a protective role

against temperature extremes, functioning in reserve due to the failure of the more temperature sensitive CN-sensitive pathway.

Nitrogen nutrition can also influence the activity of the different respiratory pathways. Barneix et al. (1984) found increased respiration in wheat roots was due to high ammonium nitrogen which was partially mediated by the CN-resistant pathway, whereas nitrate fertilized plant respiration was mediated through the CN-sensitive pathway. Lambers (1980) suggested that plants grown with an ammonium nitrogen source required less respiratory cost in the roots compared to plants fertilized with nitrate since nitrogen is incorporated in the reduced form. Since nitrate nitrogen must be reduced in the roots, more carbohydrates are required for respiratory energy costs, compared to nitrate reduction in leaves which can utilize photosynthetic energy. Blacquiere et al. (1987), using two different species of Plantago, found that roots of plants grown with an ammonium nitrogen source respired at a higher rate than those grown with nitrate fertilizer. Respiration via the CN-sensitive pathway was also slightly elevated in ammonium fertilized plants. Blacquiere (1987) experimentally determined that root growth respiration was higher in ammonium compared to nitrate fertilized plants. Ammonium was found to be a more expensive nitrogen source for the roots because of higher costs of protein and amide synthesis in the roots and because of transport costs to the shoot. When the cost of glucose equivalents was factored into the total energy cost, nitrate assimilation in the roots was more costly than for ammonium. More ATP was generated in ammonium fertilized roots. This was accomplished by increased activity of both the cytochrome and alternative respiratory pathways. Since the cytochrome path could not match the increased energy requirements, the alternative path was increased to meet metabolic demands. Therefore, it has been proposed that the CN-resistant pathway functions as a "overcharge overflow" (de Visser et al., 1986) compared to the energy-overflow hypothesis proposed by Lambers (1982).

Photosynthesis

RuBisCO

Photosynthesis is controlled by a variety of environmental and biochemical factors. One of the primary factors appears to be the biochemical regulation of CO_2 fixation by the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO). The enzyme consists of eight pairs each of large subunits (L) and small subunits (S) with a quaternary structure of L_8S_8 (Incharoensakdi and Takabe, 1987). Subunit L is considered to be the catalytic site for the activity of both the carboxylase and oxygenase functions. The large subunit (L) is encoded by the chloroplast while the small subunit (S) is nuclear encoded.

RuBisCO is activated in light by CO_2 and Mg^{2+} to form the ternary complex E-CO_2-Mg^{2+}. The ternary enzyme complex can then bind to the transition-state analog 2-carboxy-D-arabinitol-1,5-bisphosphate (CABP) to form the quaternary complex E-CO_2-Mg^{2+}-CABP. Variation in light intensity, which causes differences in stromal pH and Mg^{2+} levels, has been shown to regulate the activation of this enzyme (Seeman, 1986).

Different species vary in regards to night/day inhibition of RuBisCO (Servaites et al., 1986). Vu et al. (1984) concluded that with many plants, especially C_3 and CAM species, a large portion of RuBisCO was in an inactivatable state which did not respond to CO_2 or Mg^{2+}, but activation occurred upon exposure to light. The diurnal variation expressed by this enzyme was not due to light-induced fluxes of Mg^{2+}, but rather was caused by the nocturnal inhibitor 2-carboxy-D-arabinitol-1-phosphate, CA 1-P (Gutteridge et al., 1986). The amount of CA 1-P bound to the enzyme was high during periods of darkness and low irradiance and was not detectable during periods of high irradiance, thus showing light-dependant control. Salvucci and Anderson (1987) concluded the modulation of the activation state of RuBisCO in tobacco was mechanistically different from changes in total activity. They suggested that light dependant changes in the activation state of both CA 1-P species, as well as non-inhibitor species, were mediated by RuBisCO activase and that this regulatory protein accounted for the activation of

RuBisCO under physiological conditions. Streusand and Portis (1987) reported that RuBisCO could be activated by RuBisCO activase and ATP in the presence of RuBP and thylakoid membranes and light was not required in the system. They suggested that RuBisCO activase activity was dependant upon ATP and under in vivo conditions, ATP from photophosphorylation could act as either a regulatory metabolite or a substrate for RuBisCO activase which led to light-dependant RuBisCO activation.

RuBisCO activity was influenced by high temperatures and correlated positively with photosynthetic activity (Downton and Slayter, 1972; Laing et al., 1974; Pearcy, 1977). The thermotolerance of plants correlated positively with thermostability of RuBisCO (Weber et al., 1977; Gerwick and Williams, 1983). After exposing ivy leaves to supraoptimal temperatures for 30 minutes, Bauer and Senser (1979) found RuBisCO to be an extremely heat stable enzyme which was only inactivated when leaves were necrotically damaged at 52 C. RuBisCO activity was not significantly reduced after heat stress treatments up to 50 C. At 52 C the activity of the enzyme was not immediately affected, but RuBisCO activity corresponded to the rate of necrosis which occurred over several days. The authors concluded that due to the thermostability of RuBisCO, thermal inactivation of the enzyme at supraoptimal temperatures could not account for decreases in photosynthetic rates.

RuBisCO activity decreased over time in response to supraoptimal leaf temperatures in wheat (Al-Khatib and Paulsen, 1984). The decrease in RuBisCO activity was similar to decreases seen in photosynthetic rates, though the changes in RuBisCO activity were smaller in magnitude. Badger et al. (1982) found the activity of RuBisCO from oleander plants grown at 20 C to be 1.49 times greater than for plants grown at 45 C. However, the results indicated that RuBisCO activity at 20 C may limit photosynthesis in plants grown at 20 C compared to those grown at 45 C. For Opuntia plants grown at 20/15 and 35/15 C (day/night), the specific activity of RuBisCO was significantly higher in plants grown at 20/15 C (Gerwick and Williams, 1979). The temperature response for RuBisCO activity was similar for the two growth temperatures,

having a broad optimum between 40 to 50 C. The greatest activity of the enzyme occurred at warm temperatures, which suggested that while warm (35/15) grown plants produce less enzyme, it did not affect carbon fixation activity. The higher specific activity for RuBisCO in plants grown at 20/15 C may have been necessary to compensate for carbon fixation at cooler temperatures. Elevated temperatures also caused greater inactivation of RuBisCO in wheat leaves compared to controls (Grover et al., 1986).

Monson et al. (1982) suggested that heat stress caused alterations in RuBisCO structure which altered its affinity for CO_2. Weis (1981) proposed that RuBisCO activity in vivo required a form of activation which involved properties of the thylakoid membrane which were affected by heat treatments. Since changes in thylakoid membrane properties and RuBisCO activity occurred at temperatures which were considered to be physiological, and these changes were reversible, it was suggested that these two properties played a role in the temperature regulation of the overall photosynthetic process. It was concluded that RuBisCO was the primary site of heat inactivation since RuBP (substrate) levels accumulated while PGA (product) levels decreased. Weidner and Fehling (1985) concluded that high temperature increased wheat RuBisCO solubility. Differences in the tertiary structure of the enzyme at 36 C compared to 4 or 20 C caused an increase in the hydrophobicity/solubility of RuBisCO. RuBisCO was found to have an increased hydrophilic protein surface with reduced accessibility of R-SH groups when grown at 36 C. Differences were discussed in terms of the stabilizing effect of RuBP binding on conformational stability. Amino acid composition was not significantly altered.

Hall and Keys (1983) suggested that photosynthetic and photorespiratory response to temperature was due more to the relative solubilities of CO_2 and O_2 than to changes in the kinetic parameters of reactions catalyzed by RuBisCO. This agreed with the research of Ku and Edwards (1977). Markus et al. (1981), using a heat tolerant (Saladette) and a heat sensitive (Roma VF) cultivar of tomato, studied the effects of high temperature on RuBisCO activity and photosynthesis. One hour in vitro exposures to 50 C decreased the activity of RuBisCO by 75%

in Roma VF, while no effect was detected for the heat tolerant 'Saladette'. RuBisCO activity decreased in both cultivars after a 24 hour exposure at 35 to 40 C, but recovered at lower temperatures. The heat tolerant cultivar, 'Saladette', demonstrated a smaller reduction in photosynthesis and mesophyll resistance increased less compared to 'Roma VF'.

Carbon Dioxide Assimilation

Environmental stresses can decrease photosynthesis by limiting the photosynthetic efficiency of the mesophyll or by limiting CO_2 conductance through stomatal changes. Stomatal limitation of CO_2 assimilation often decreases in response to environmental stresses (light, nutrition and chilling), indicating that stomata generally function to minimize water loss while only marginally limiting carbon assimilation (Farquhar and Sharkey, 1982). For C_3 plants, non-stomatal limitations of photosynthesis caused by environmental stresses may be analyzed by looking at the relationship between assimilation rate (A) and leaf intercellular CO_2 concentration (c_i). The nature of A-c_i curve responses in woody plants has not been studied extensively (Davis et al., 1987), particularly in regards to environmental stress.

Farquhar and Sharkey (1982) described a method for studying the relationships between conductance and CO_2 assimilation. Net assimilation (A) is related to C_a and C_i, the partial pressures of CO_2 in the atmosphere and internal leaf spaces, respectively. For such equations it is necessary to use the mol or volume fractions of C_a and C_i, thus c_a and c_i are used. Internal CO_2 partial pressure (c_i) may be calculated as:

$$c_i = c_a - A/g'$$

where g' is the conductance for water vapor from the mesophyll tissue.

By measuring A while changing external CO_2 concentrations, an A-ci curve can be generated which is independent of stomatal conductance. This allows for the analysis of relationships between metabolism and CO_2 supply (Lawlor, 1987). By plotting A against c_i, a relationship can be generated which describes the demand function, the dependance of the rate of CO_2 assimilation on the partial pressure of CO_2 at the sites of carboxylation, and a

supply function, an equation describing the gaseous diffusion of CO_2 from the atmosphere to the intercellular spaces. The equation describing the supply function is:

$$A = g' (c_a - c_i)$$

At low c_i concentrations, RuBisCO is saturated in respect to its substrate, RuBP. The initial effect of increasing c_i will be the activation of RuBisCO. This is followed by a linear response of assimilation rate to CO_2 pressure at the site of carboxylation, C_c. This linear portion of the response curve is RuBP saturated and carboxylation is limited due to low CO_2 partial pressures. At higher c_i concentrations, carboxylation becomes saturated and the ability to regenerate RuBP becomes limiting. The initial slope of the line in the RuBP saturated area should be linearly related to RuBisCO activity in the leaf and depends solely upon the kinetic parameters of the enzyme (von Caemmerer and Farquhar, 1981).

Internal leaf resistance to CO_2 fixation at high temperatures was centered in the mesophyll tissue (Hofstra and Hasketh, 1969) which included rate limitations imposed by the diffusion of CO_2 into the cells and the biochemical processes of fixation. Mesophyll resistance (r_m) involved the diffusion of CO_2 in the liquid phase through the cell wall, cytoplasm and the chloroplast stroma (Gaastra, 1959). Farquhar and von Caemmerer (1982) concluded that c_c was very close to c_i and therefore r_m can generally be ignored for most photosynthetic modelling purposes.

Varied results can be found in the literature regarding stomatal conductance, mesophyll resistance and intercellular CO_2 in response to heat stress. Many plants open their stomata when subjected to high temperatures (Babuskin and Barabal'chuk, 1974). This allows plants to survive air temperatures due to transpirational cooling which would otherwise be lethal if the stomata remained closed. Using tobacco, Zioni and Itai (1972) found decreased CO_2 fixation at 45 C was not due to increased stomatal resistance. They proposed that membrane damage or decreased enzyme activity was the cause for decreased CO_2 fixation. Bauer (1978), working with English ivy, found a non-stomatal inhibition of photosynthesis as well as an increased CO_2 compensation point after heat stress. No difference in mesophyll resistance was noted in heat-

stressed leaves. The stomates of heat-stressed leaves were opened wider than in non-stressed leaves (Bauer, 1979). A reduction in stomatal conductance was caused by increased c_i due to reduced photosynthetic activity. Monson et al. (1982) found stomatal conductance had no significant role in determining the temperature response of photosynthesis in a C_3 grass. However, intercellular CO_2 conductance increased over the entire experimental temperature range (10 to 50 C) when the vapor pressure deficit was maintained constant. Using the desert plant Atriplex lentiformis, Pearcy (1977) determined that leaf CO_2 conductance was not an important factor in controlling the temperature dependance of net CO_2 uptake or photosynthetic capacities at any temperature. At high temperatures (>40 C), improved photosynthetic rates appeared to be due to decreased respiration rates, a decreased temperature dependance of respiration and increased thermal stability of photosynthetic CO_2 exchange mechanisms.

The CO_2 response of photosynthesis in Quercus suber during high midday temperatures was found to be a strong stomatal closure which occurred along with simultaneous suppression of net CO_2 uptake (Tenhunen et al., 1984). The carboxylation efficiency at 38 C was only 30 to 40% of that found at 25 C. Photosynthetic rates decreased in two tomato cultivars when the plants were exposed to temperatures of 35 to 40 C for varying lengths of time. Increased temperature increased transpiration. Decreased photosynthesis caused by exposure to high temperature were due to increased mesophyll resistance and to a lesser degree, increased stomatal resistance to CO_2 diffusion. The ratio between stomatal resistance and mesophyll resistance after heat stress for both cultivars was 1.5 to 1.6. Stomatal resistance was not affected by temperatures of in the range of 24 to 35 C.

Supraoptimal root-zone temperatures have been shown to decrease photosynthetic rates (Johnson and Ingram, 1984; Foster, 1986; Ramcharan, 1987). Working with 'Rotundifolia' holly, Foster (1986) found root-zone temperatures above 32 C decreased canopy photosynthetic rates. However, root-zone temperature had no effect on transpiration rate. Therefore, increased stomatal resistance was dismissed as a possible cause for decreased photosynthetic rates at

root-zone temperatures above 28 C. This supported the idea of non-stomatal inhibition of photosynthesis induced by supraoptimal root-zone temperatures.

Chlorophyll Fluorescence

Advances in the measurement of heat damage to photosynthetic tissues came with the introduction of chlorophyll *a* fluorescence emission techniques (Schreiber and Berry, 1977; Schreiber and Armond, 1978). At room temperature, light excites chlorophyll which causes fluorescence to be emitted from chlorophyll *a* of PSII in the wavelength range of 680 to 720 nm. This fluorescence can then be detected using a fast responding detector. Unfavorable environmental stresses often cause inhibition of photochemical processes which control in vivo fluorescence yields. Chlorophyll *a* fluorescence can be used to analyze interactions between light harvesting, electron transport, CO_2 fixation, and NADPH and ATP synthesis concomitant with O_2 evolution and CO_2 assimilation (Lawlor, 1987). Chlorophyll fluorescence has become a simple method for screening a large number of plants for their responses to environmental stresses such as cold, heat and drought (Smillie and Hetherington, 1983; Havaux et al., 1988).

Several fluorescence parameters can be obtained by measuring chlorophyll *a* fluorescence at room temperature. F_o refers to non-variable or initial fluorescence and designates the fluorescence level when all the reaction centers of PSII are open. F_m is the maximal fluorescence which occurs at the P-peak after a saturating pulse of light which causes the transient closing of the reaction centers. F_v, or variable fluorescence, is equal to $F_m - F_o$ and F_v gives an indication of the quantum yield of photochemistry. F_v/F_m estimates the photochemical efficiency of PSII. Photochemical quenching (q_Q) can be calculated as follows:

$$q_Q = (F_m - F_v)/(F_m - F_o)$$

Smillie and Hetherington (1983) used F_r, the maximal rate of the induced rise in chlorophyll fluorescence, to measure and compare the responses of leaf tissue to various environmental stresses.

When leaves are placed under conditions which caused heat stress, increased F_o was recorded (Schreiber and Berry, 1977; Smillie and Nott, 1979). The critical temperature (T_c) at which F_o increased was correlated to plant heat tolerance. (Smillie and Nott, 1979; Bilger et al., 1984; Havaux et al., 1988). The rise in F_o which occurred when T_c was reached was associated with the dissociation of PSII reaction centers from the light-harvesting (LHCP) apparatus (Armond et al., 1979; Sundby et al., 1986). PSII and the water-splitting complex are particularly sensitive to high temperature stress (Berry and Bjorkman, 1980). Freeze-fracture studies have shown that loss of granal stacking and changes in the distribution of intramembrane particles believed to be associated with PSII occurred in heat-stressed chloroplasts (Armond et al., 1980; Gounaris et al., 1984). These changes may have been related to phase separations of non-bilayer lipids which occurred in heat-stressed chloroplasts (Gounaris et al., 1983). Fluorescence (F_r) has been used as a technique for the assessment of differences in heat tolerance of clones from different environments (Hetherington et al., 1983) and differences between temperate and tropical crops (Smillie and Hetherington, 1983).

Heating of pea, barley and wheat leaves to temperatures above 37 C decreased F_v, which reflected a suppression of PSII activity (Bukhov et al., 1987). PSI was shown to be more resistant to heat stress damage than PSII. Exposure of chloroplasts to temperatures above 50 C led to increased chlorophyll *a* fluorescence associated with PSI activity (Downton and Berry, 1982). Mannan et al. (1986) reported a species-specific secondary rise in fluorescence in the temperature range of 65 to 70 C. This species-specific response could not be explained by changes between the two photosystems or by chlorophyll *a* fluorescence associated with PSI at high temperatures. Exposure of chloroplasts to high temperature stress also increased susceptibility of chlorophyll to photobleaching processes (Gounaris et al., 1983). Williams et al. (1986) found increased susceptibility of PSI pigments to photobleaching were due to interruption in the flow of reductants from PSII to PSI which normally protected PSI from photooxidation.

CHAPTER 2

THE INFLUENCE OF SUPRAOPTIMAL ROOT-ZONE TEMPERATURES ON ^{14}C-PHOTOSYNTHATE PARTITIONING IN *ILEX CRENATA* THUNB. 'ROTUNDIFOLIA'

Introduction

Supraoptimal root-zone temperatures reduce the growth of container-grown plants. Temperatures as high as 58 C have been recorded in media of container-grown plants in Florida (Martin & Ingram, 1988). Ingram et al. (1988) reported reductions in growth and changes in root distribution of *Ilex crenata* 'Helleri' when containers were exposed to direct radiation (average maximum of 50 C in the western quadrant) compared to plants in shielded containers (average maximum of 42 C in the western quadrant). Laboratory experiments have shown that roots of 'Helleri' holly were injured by a 30 minute exposure to 51 C (Ingram, 1986).

Foster (1986), working with *Ilex crenata* 'Rotundifolia', found more ^{14}C-labelled photosynthate in leaf tissue and less in the root fraction when root-zone temperature was maintained at 28 compared to 40 C. He hypothesized that 1) the decrease of labelled ^{14}C in the roots with increasing temperature was due to a physical blockage thereby decreasing the amount of partitioned label or 2) the decrease in the root was due to an increase in transport of labelled assimilates to the roots with subsequent loss of label via root respiration at increased root-zone temperatures.

Environmental conditions which restrict plant growth alter the distribution of carbon between plant and soil fractions and carbon loss through respiration (Whipps,1984). Roots are dependant upon carbon assimilates imported from the shoot. Loss of carbon compounds from roots is influenced by several environmental factors, including temperature (Lambers, 1987). Import rate

is determined by the utilization of assimilates in the sink organ. Wardlaw (1968) suggested that differences in development between shoots and roots may be due to competition for available carbon at high temperatures. The objectives of this study were to determine: 1) if a portion of the root system of Ilex crenata 'Rotundifolia' grown at near optimum temperatures could compensate in terms of growth for a portion of the root system exposed to temperatures above optimum, 2) if heating only a portion of the root system would alter the pattern of photosynthate partitioning to different plant parts, and 3) if high root-zone temperatures caused increased loss of current photoassimilates through root respiration.

Materials and Methods

Stem-tip cuttings of Ilex crenata Thunb. 'Rotundifolia' were taken during the first week of September, 1987 and rooted in a mixture of 80% perlite - 20% sphagnum peat moss under intermittent mist for a period of twelve weeks. Rooted cuttings were potted into paired 500 ml square plastic pots containing Metro-Mix 300 (W.R. Grace and Co., Cambridge, MA) with approximately one-half of the roots in each container. After twelve weeks the split-root plants were transplanted into paired 1200 ml clear plastic bags containing 1100 cm^3 washed quartz sand. Each bag was then placed into polyvinyl chloride cylinders (8x24 cm) which provided light exclusion and physical support. After a six-week growth period, the plants were moved from the greenhouse to a growth room. Experiments were initiated one week later and were conducted in a 3.0 m by 7.6 m walk-in growth room. Irradiance was supplied by 18 1000-W phosphor coated metal-arc HID lamps (GTE Sylvania Corp., Manchester, NH). A photosynthetic photon flux density of 1000 $\mu mol\ s^{-1}\ m^{-2}$ was measured at canopy height using a quantum radiometer (LI-COR Inc., Lincoln, NE). The photoperiod was 13 hours (0500 to 1800 HR) with the dark period interrupted for three hours (2200 to 0100 HR) by incandescent light. Air temperature was maintained at 28±1 C during the light period and 21±1 C during the dark period. Relative humidity was held at approximately 40% during the light period and 90% during

the dark period. Plants were watered as needed and fertilized twice weekly (200 ml) with a 300 mg L^{-1} N solution of soluble 20N-8.8P-16.6K fertilizer (Peters 20-20-20, W.R. Grace and Co., Cambridge, MA).

Root-zone temperature combinations for the split-root systems (left/right) were 30/30, 30/34, 30/38, 30/42, 34/34, 38/38, and 42/42 C. Treatments were imposed for 21 days in a randomized complete block design replicated six times over an eight week period. Root-zone temperatures were maintained at ±1 C using an electronically controlled root-heating system described by Foster (1986). Shoot length and branch number was recorded on days 1 and 21. On day 21 each plant was moved to an assimilation chamber for labelling studies.

Assimilation Chamber

A split-root assimilation chamber consisting of three separate plexiglass compartments was designed for $^{14}CO_2$-labelling the foliar portion of a plant while allowing the collection of respired ^{14}C from the separate split-root halves maintained at treatment temperatures. Each compartment was cemented with methylene chloride and sealed with silicon rubber to prevent leaks. The foliar compartment measured 14 cm by 15 cm by 28.5 cm (L x W x H). Each split-root half compartment measured 14 cm by 14 cm by 28 cm (L x W x H). Each of the three compartments had an additional volume measuring 9 cm by 6 cm by 19 cm (L x W x H) to accommodate a circulating fan. Each box fan (Dayton Electric Mfg. Co., Chicago, IL), 7.9 cm by 3.8 cm by 7.9 cm (L x W x H), circulated air in the chamber at a rate of 46 m^3 hr^{-1}.

$^{14}CO_2$ - Labelling

Each plant was transported to the laboratory and sealed inside the assimilation chamber between 0730 and 0800 HR. Root-zone temperatures were electronically controlled as described by Foster (1986). A high pressure sodium lamp (Lumalux 400W, GTE Sylvania Inc., Manchester, NH) was used to provide a photosynthetic photon flux density of 700 to 800 μmol s^{-1} m^{-2} at canopy level. Shoot and root-zone temperatures were monitored using a digital thermocouple thermometer (Cole Palmer Instrument Co., Chicago, IL). Shoot temperatures ranged from 30

±1 C at the beginning of the labelling period to 32.5±1 C six hours later. Relative humidity was monitored using an Airguide hygrometer (Chicago, IL) and was maintained at 64±3%.

The shoot chamber and air flow system were flushed with N_2 gas to zero the infrared gas analysis system (Anarad Model AR-600R, Anarad Inc., Santa Barbara, CA). Each plant was then sealed in the chamber and flushed with N_2 for two minutes (CO_2 levels of approximately 150 to 200 $\mu l\ L^{-1}$) before filling the shoot system with 311 $\mu l\ CO_2\ L^{-1}$. The system was then pulsed for 60 sec with 15.3 $\mu Ci\ L^{-1}\ ^{14}CO_2$. Total CO_2 uptake was monitored for 30 min using a closed system before another 60 sec pulse of $^{14}CO_2$ was added. After the 1 hr pulse period, the shoot chamber was flushed with N_2 followed by a 5 hr chase period using ambient air (380±20 $\mu l\ CO_2\ L^{-1}$). A circulating pump (Neptune Dyna-Pump, Universal Electric Co., Owosso, MI) and Dwyer flow meters (Dwyer Instruments, Inc., Michigan City, IN) were used to control the flow rate through the shoot compartment system at 1.5 L min^{-1}.

Air was pumped through each of the root compartments during the 6 hr period at a rate of 1.0 L min^{-1} after being bubbled through 400 ml of 0.2 N NaOH to reduce the CO_2 concentration to approximately 10 $\mu l\ CO_2\ L^{-1}$. The background level of CO_2 accounted for less than 5% of the total CO_2 trapped. Air exiting each root compartment was bubbled through 200 ml of 0.2 N NaOH to trap respired CO_2.

Sampling and Analysis

$^{14}CO_2$-labelled plants were separated into leaves, stem and root halves before being weighed. Samples were frozen in liquid N_2, boiled in 80% (v/v) ethanol for two hours and then ground in a Virtis homogenizer (The Virtis Company, Inc., Gardiner, NY). Leaf area was estimated using a prediction equation based on leaf fresh weight (R^2=0.99, leaf area = 5.20(leaf fresh weight) + 39.37). Ethanol soluble material was separated from ethanol insoluble material by filtering the ground extracts through filter paper and thoroughly washing with ethanol. Samples of ethanol soluble material (50 μL) were added to 10 ml of scintillant (ScintiVerse II, Fisher Scientific, Orlando, FL) and 3 ml of distilled H_2O. Ethanol insoluble

material was oven-dried for 5 days at 63°C before adding a 0.02 g sample to scintillant. Label in the potting medium was determined by boiling 10 cm^3 samples in ethanol.

Excess $BaCl_2$ was added to the NaOH traps to precipitate the carbon dioxide as $BaCO_3$. The precipitate was allowed to settle overnight, collected in centrifuge tubes, washed with deionized water, centrifuged for 5 minutes at 2200 rpm and dried at 63 C for 7 days before analysis. The dried $BaCO_3$ was then ground into a fine powder and 0.05 g samples were added to 10 ml of scintillant with 3 ml of distilled H_2O and were analyzed via liquid scintillation spectrophotometry to quantify respired $^{14}CO_2$. Total respired CO_2 (plant plus microbial) was calculated from the total $BaCO_3$ collected as precipitate.

Radioactivity was determined via liquid scintillation spectrophotometry using a LKB 1214 liquid scintillation counter (LKB Wallac, Turku, Finland). Labelled carbon in the leaves, stems, root halves, growth medium and carbon respired from the roots was expressed as a percentage of the total labelled carbon recovered from the plant. The data were analyzed as a general linear model (Statistical Analysis System, Raleigh, N.C.) with orthoganol contrasts designed to address research objectives.

Results and Discussion

Growth Analysis

Growth values for the above-ground portions of Ilex crenata 'Rotundifolia' decreased as temperature increased when both root halves were heated in relation to the control (30/30) (Table 1). Leaf area (R^2=0.84), leaf fresh weight (R^2=0.84) and shoot elongation (R^2=0.66) decreased linearly with increasing root-zone temperatures from 30 to 42 C when both root halves were at the same temperature. Growth and morphological responses known to be influenced by root-zone temperatures include plant height, leaf expansion, shape and color, root extension, branching, root and shoot dry weight, flowering and fruiting (Cooper,1973). Growth generally increases to an optimum temperature and then decreases as the temperature rises.

Table 2-1. Effects of root-zone temperature treatment of split-root systems on selected growth parameters of Ilex crenata 'Rotundifolia' after 21 days.

Treatment (Left/Right)	Leaf Area (cm^2)	Shoot Elongation[z] (cm)	Fresh Weight (g)				
			Leaf	Shoot	Total Root	Left Root	Right Root
30/30	302	37	7.5	5.8	16.4	8.8	7.6
30/34	257	35	6.4	5.2	13.3	9.1	4.2
30/38	324	28	8.1	5.7	17.2	10.7	6.5
30/42	225	14	5.6	4.6	13.5	8.1	5.4
30/30	302	37	7.5	5.8	16.4	8.8	7.6
34/34	219	25	5.4	4.2	10.3	4.6	5.7
38/38	215	11	5.3	4.1	9.8	5.6	4.2
42/42	208	13	5.2	4.4	11.1	4.9	6.2
Contrasts							
30/30 vs 30/34	ns	ns	ns	ns	ns	ns	*
30/30 vs 30/38	ns	ns	ns	ns	ns	ns	ns
30/30 vs 30/42	*	*	*	*	ns	ns	ns
30/34 vs 34/34	ns	ns	ns	ns	ns	**	ns
30/38 vs 38/38	ns	**	**	**	**	**	ns
30/42 vs 42/42	ns	ns	ns	ns	ns	*	ns

ns, *, ** Nonsignificant or significant at P=0.05 or 0.01, respectively.

[z] - Mean initial shoot length was 52 cm.

Leaf area, shoot elongation and leaf and stem fresh weight decreased when one-half of the root system was exposed to 42 C for three weeks compared to the control (Table 1). Stem fresh weight was decreased by the 30/42 and 38/38 treatments compared to 30/30 and 30/38, respectively. For leaf area and leaf and stem fresh weights, these results show that half of the root system in 'Rotundifolia' holly exposed to 38 C was damaged less compared to the detrimental effects of the whole root system being exposed to 38 C. However, the roots exposed to 30 C could not overcome the damage resulting from exposure of the other root half to 42 C.

The only significant difference detected for total root fresh weight was between the 30/38 and 38/38 treatments (Table 1). The 30/38 treatment had 1.8 times more root fresh weight. The ratio of left root fresh weight to right root fresh weight was not significantly influenced by treatment (data not shown). Heating the right root half above 30 C did not influence the fresh weight of the left root half.

The optimum temperature for root elongation has been considered to be between 20 to 30 C for most plants. In contrast, Harrison (1989) found no difference in the dry weight gain of Ilex crenata 'Rotundifolia' when grown at root-zone temperatures of 28, 34 and 40 C for six weeks. Root-zone temperature up to 40 C for six hours daily did not affect shoot or root dry weight for ixora or citrus, but the 40 C treatment decreased the root:shoot ratio after ninety days (Ingram et al., 1986a). Barr and Pellet (1972) found decreases in root dry weight of five woody species in response to constant root-zone temperatures up to 40 C for sixty days.

The partitioning of photoassimilates between shoots and roots is influenced by root-zone temperatures and this can often be seen as differences in root:shoot ratios at increased root-zone temperatures (Cooper, 1973; Johnson and Ingram, 1984; Ramcharan, 1987). Although differences were found in this study for individual growth parameters, no differences were detected among treatments for root:shoot ratios (data not shown). Total plant biomass ranged from 19.2 g (38/38) to 31.0 g (30/38). Therefore, while temperature influenced the overall size

of the plants, the root:shoot ratios remained the same. Ingram et al. (1988), using different container spacing strategies to study the influence of temperature on Ilex crenata 'Helleri' growth through a growing season, found decreased root growth and subsequently decreased root:shoot ratios associated with elevated root-zone temperatures.

Distribution of ^{14}C-Photosynthates

Photosynthetic rates were measured during the first 30 minutes of the labelling period. Although photosynthetic rates ranged from 2.3 (30/30) to 3.6 μmol CO_2 s^{-1} m^{-2} (38/38), no differences were detected due to root-zone temperature treatment. Foster (1986) found that shoot carbon exchange rates decrease in 'Rotundifolia' holly when grown at root-zone temperatures of 36 and 40 C for one week. Other researchers have also reported decreases in photosynthetic rates in response to long-term exposure of woody plants to supraoptimal root-zone temperatures (Gur et al., 1972; Johnson and Ingram, 1984). Elevated demands by sink tissues for assimilates can increase photosynthetic supply by raising rates of CO_2 fixation (Gifford and Evans, 1981).

For total ^{14}C recovered in the leaf or root fractions (Table 2), no significant differences were detected among treatment comparisons, but differences were detected for total recovered ^{14}C in the stem fraction. Percent ^{14}C in the stem for the 30/34, 30/38 and 30/42 treatments decreased compared to the 30/30 control. Because no differences were detected for the percent labelled photosynthate remaining in the leaves, this may have indicated that the roots were dependant upon a current supply of assimilates available from the stem at most temperature/split-root treatment combinations.

A greater portion of the ^{14}C in the stem was in the insoluble fraction for the 30/38 treatment (28.5%) than for the 38/38 (20.7%) treatment (Table 2). This effect was also evident when data were expressed on a fresh weight basis. A greater demand for readily available assimilates in the rapidly respiring roots was indicated compared to the stem since there was a corresponding increase in the soluble stem fraction at 38/38.

Table 2-2. Percent of recovered ^{14}C partitioned to leaf, stem and total root (both root halves) as influenced by root-zone temperature treatments. Plants were pulsed with $^{14}CO_2$ for one hour followed by a five hour chase period. The percent ^{14}C partitioned to the stem is also expressed as the percent occurring in the soluble and insoluble fraction. Each value is the mean of six replicate plants.

Treatment (Left/Right)	Leaf	Stem	Stem (%Soluble)	Stem (%Insoluble)	Root
30/30	71.0	16.1	77.4	22.6	12.9
30/34	71.6	12.3	73.6	26.4	16.1
30/38	70.5	11.9	71.5	28.5	17.6
30/42	74.9	10.3	80.9	19.1	14.8
30/30	71.0	16.1	77.4	22.6	12.9
34/34	77.2	11.2	71.1	28.9	11.6
38/38	68.9	8.8	79.3	20.7	22.3
42/42	71.6	11.2	81.8	18.2	17.2
Contrasts					
30/30 vs 30/34	ns	*	ns	ns	ns
30/30 vs 30/38	ns	**	ns	ns	ns
30/30 vs 30/42	ns	**	ns	ns	ns
30/34 vs 34/34	ns	ns	ns	ns	ns
30/38 vs 38/38	ns	*	*	*	ns
30/42 vs 42/42	ns	ns	ns	ns	ns

ns,*,** Nonsignificant or significant at P=0.05 or 0.01, respectively.

Table 2-3. Effect of root-zone temperature treatments (21 days) on the allocation of total below-ground ^{14}C assimilates between the root, respired $^{14}CO_2$ and the potting medium after a one hour pulse followed by a five hour chase period. The data presented is the total for both root halves and each value is the mean of six replicate plants.

Treatment (Left/Right)	Root Tissue (Insoluble)	Root Tissue (Soluble)	Respired	Media
30/30	14.6	60.2	19.0	6.2
30/34	10.0	52.0	30.8	7.2
30/38	8.0	50.0	37.3	4.7
30/42	9.9	68.0	15.8	6.3
30/30	14.6	60.2	19.0	6.2
34/34	9.4	60.6	21.5	8.6
38/38	9.0	48.4	32.5	10.1
42/42	11.6	58.1	10.0	20.3
Contrasts				
30/30 vs 30/34	*	ns	ns	ns
30/30 vs 30/38	**	ns	ns	ns
30/30 vs 30/42	**	ns	ns	ns
30/34 vs 34/34	ns	ns	ns	ns
30/38 vs 38/38	ns	ns	ns	ns
30/42 vs 42/42	ns	ns	ns	*

ns,*,** Nonsignificant or significant at P=0.05 or 0.01, respectively.

Differences were detected in the proportions recovered from the insoluble fractions in the roots and in the potting media (Table 3), although no differences were detected among treatments for the percent recovered ^{14}C that went to the roots (Table 2). The percent recovered ^{14}C in the insoluble root fraction was reduced when one-half of the root system was exposed to 34, 38 or 42 C for six hours daily. No additional reduction in the percent recovered ^{14}C in the insoluble fraction was noted when both root halves received elevated temperatures. There were 3.2 times more ^{14}C recovered in the media fraction when both root halves were grown at 42 C as compared to plants grown at 30/30 and 30/42.

Temperature treatments influenced the allocation of ^{14}C-labelled photosynthates to the various fractions in each root segment (Table 4). The percentage of recovered ^{14}C in the soluble fraction of the left root half exposed to 30 C was increased by a factor of 1.7 and decreased the percent recovered from the soluble fraction of the root half exposed to 42 C. Approximately twice as much ^{14}C was recovered from the 30 C left root half of the 30/42 treatment compared to the 42/42 treatment. The treatments 30/34, 30/38 and 30/42 had approximately one-third the amount of ^{14}C in the insoluble fraction of the higher temperature right root half compared to the control (Table 4). At 42/42, the percent recovered ^{14}C in the insoluble and soluble fractions of the right root half was three and two fold greater, respectively, than found in the 42 C half of the 30/42 treatment, possibly indicating preferential partitioning to the non-heated 30 C half.

The ratio of percent total ^{14}C (insoluble + soluble) in the 30 C versus heated right root tissue rose 1.6, 2.4 and 4.3 fold as temperature on the heated right side increased from 34 to 42 C, respectively (Table 4). For the 30/30, 30/34 and 30/38 treatments, the percent recovered ^{14}C for each root half was approximately 1:1. At 30/42, this ratio was approximately 3:1. There was a difference in partitioning of total ^{14}C to each root half when only one side of the root system was grown at 42 C.

Table 2-4. Percent of total below-ground ^{14}C assimilates recovered from half-root systems of split-root plants as affected by root-zone temperature. Plants were treated for 21 days at the respective root-zone temperatures. Plants were labelled with $^{14}CO_2$ for one hour followed by a five hour chase period. Each value is the mean of six replications.

Treatment (Left/Right)	Root (Left)				Root (Right)			
	Insoluble	Soluble	Respired	Media	Insoluble	Soluble	Respired	Media
30/30	8.2	32.3	10.1	3.2	6.5	27.9	8.9	2.9
30/34	7.8	30.4	10.8	3.9	2.2	21.6	20.0	3.3
30/38	5.7	35.1	6.0	2.4	2.3	14.9	31.3	2.3
30/42	7.8	55.4	8.0	3.3	2.1	12.7	7.7	3.0
30/30	8.2	32.3	10.1	3.2	6.5	27.9	8.9	2.9
34/34	4.4	26.5	15.8	4.3	5.0	34.1	5.7	4.2
38/38	6.2	31.4	10.7	5.2	2.8	16.9	21.9	4.9
42/42	5.6	26.5	4.8	10.4	6.0	31.6	5.2	9.9
Contrasts								
30/30 vs 30/34	ns	ns	ns	ns	**	ns	ns	ns
30/30 vs 30/38	ns	ns	ns	ns	**	*	**	ns
30/30 vs 30/42	ns	**	ns	ns	**	**	ns	ns
30/34 vs 34/34	ns	ns	ns	ns	ns	*	ns	ns
30/38 vs 38/38	ns	ns	ns	ns	ns	ns	ns	ns
30/42 vs 42/42	ns	**	ns	**	**	**	ns	**

ns,*,** Nonsignificant or significant at P=0.05 or 0.01, respectively.

The only difference in amounts of respiratory $^{14}CO_2$ released from roots was for the warmed half of the 30/38 treatment compared to its 30 C counterpart in the control (30/30) (Table 4). In the 30/38 treatment, 84% of the total ^{14}C respired from both root halves came from the root half exposed to 38 C, even though the fresh weight of roots in the half at 30 C was 1.8 times greater than the root fresh weight in the half at 38 C. Total CO_2 respired (Table 5) from the left root half was 1.8 times greater for 30/38 compared to 30/30, though this difference was not evident when expressed on a weight basis. More current non-labelled photosynthates or previously accumulated photosynthates may have been respired from non-heated portion of the root system in relation to the warmer right root half, although no increase occurred in ^{14}C respired from the non-heated root half. Kouchi et al. (1986) used a compartmental analysis model to study the partitioning of photoassimilated carbon in soybean plants and found the soluble pool in roots acted differently from soluble pools in other organs. This suggested that the soluble fraction of the root can temporarily store carbon as a reserve respiratory pool. Such a partitioning scenario may be the case in the non-heated root halves and could function to compensate for increases in current respired ^{14}C and decreased soluble and insoluble carbon pools which occur in the root halves at the higher temperatures.

Increases in total CO_2 respired per gram fresh weight (Table 5) were detected between 30/30 vs. 30/34 and 30/38 vs. 38/38 for the warmer right root half. No differences were detected for total CO_2 respired from the right root half for any of the treatment comparisons. Increases in current ^{14}C respired could indicate a corresponding rise in the demand for readily available respiratory substrates which in turn is likely to correspond to decreased insoluble and soluble photosynthates in the right root segments. Recently fixed carbon has been found to dominate the carbon released by plant roots as respired CO_2 (Meharg and Killham, 1988). Since no differences were detected for total CO_2 respired from the higher temperature right root halves, this implied that long-term increases in root-zone temperature from 30 to 42 C were not reflected in rates of respiratory CO_2 loss at the end of the three week period. The short-term

Table 2-5. Effect of root-zone temperature treatment on the total CO_2 respired from each half-root system of a split-root plant.

Treatment (Left/Right)	mg CO_2 hr^{-1} (Left)	mg CO_2 hr^{-1} (Right)	mg CO_2 hr^{-1} g^{-1} FW (Left)	mg CO_2 hr^{-1} g^{-1} FW (Right)
30/30	18.5	21.5	2.33	2.83
30/34	28.5	25.0	3.67	6.83
30/38	32.8	21.3	4.17	3.33
30/42	23.8	22.3	4.33	6.00
30/30	18.5	21.5	2.33	2.83
34/34	22.7	18.0	6.17	3.16
38/38	24.5	24.5	5.17	7.50
42/42	28.0	24.5	6.00	4.67
Contrasts				
30/30 vs 30/34	ns	ns	ns	*
30/30 vs 30/38	*	ns	ns	ns
30/30 vs 30/42	ns	ns	ns	ns
30/34 vs 34/34	ns	ns	ns	*
30/38 vs 38/38	ns	ns	ns	*
30/42 vs 42/42	ns	ns	ns	ns

ns,*,** Nonsignificant or significant at P=0.05 or 0.01, respectively.

response of respiration rates in actively growing tissues is to double with each 10 C increase in temperature up to 45 C. During a 24 hour period, Shishido et al (1987) found about 75% of the imported labelled carbon to be lost from cucumber roots as temperature increased from 15 to 30 C. Although total CO_2 respired from the left (30 C) root was increased by the 30/38 treatment compared to 30/30, there was no difference in the amount of currently labelled ^{14}C respired from this portion of the root. More currently labelled ^{14}C was respired from the 38 C right root half of the 30/38 treatment but the total respired CO_2 did not differ between halves. Therefore, the portion of the root system at 38 C acted as a greater sink for current photosynthate than the root half at 30 C. This indicates that the unheated root half at 30 C respired more previously fixed carbon.

Increased root-zone temperatures have been shown to increase the maintenance portion of root respiration in sunflower (Szaniawski and Kielkiewicz, 1982). Supraoptimal temperatures are known to increase maintenance respiration while causing decreases in growth due to depletion of the available carbohydrate pool (Gent and Enoch, 1983). Increased maintenance respiration with a corresponding decrease in growth respiration due to greater maintenance costs at higher root-zone temperatures could account for decreased root fresh weights (Table 1) and shifts in ^{14}C partitioned to soluble fractions (Table 4). Lambers (1987) has suggested that 15 to 30% of all photosynthates are respired by roots with an estimated 10% of daily photosynthesis going towards maintenance costs of the roots. The warmer right root half of the 30/38 treatment respired 5.5% of the total ^{14}C recovered from the plant after a six hour labelling period (Tables 2,3 and 4). Respired ^{14}C from both root halves of the 38/38 treatment accounted for 7.3% of the total recovered ^{14}C. These respiration values are in line with those projected by Lambers (1987) if respiration values are considered over a 24 hour period.

Compared to the 30/42 treatment, a significant increase was detected in the root ^{14}C media fractions for the left and right root halves in the 42/42 treatment. Loss of cellular components can increase with increasing temperatures due to loss of membrane integrity (Levitt, 1980).

Critically high temperatures leading to 50% electrolyte leakage from root cell membranes of various woody plants decreased linearly as exposure time increased exponentially (Ingram, 1985; Ingram, 1986; Ingram et al., 1986b). The predicted critical temperature for Ilex crenata 'Rotundifolia' after a 30-min exposure was 48±1.5 C (Chapter 3). Foster (1986), using nonacclimated 'Rotundifolia' holly liners, found root-zone temperatures of 45 C for six hours daily to be lethal within 48 hours. It appears that a three week exposure of both root halves to 42 C caused enough membrane damage to increase the loss of labelled photosynthates into the growth medium. Preferential partitioning of photosynthates to the non-heated left root half in the 30/42 treatment may account for the lack of ^{14}C found in the media fraction from the 42 C right root half for this treatment.

Photosynthates which are transported to the roots may be utilized by a variety of carbon sinks which include exudation, growth and respiratory processes. The loss of carbon by exudation is generally considered to be 5% or less of the total partitioned photosynthate, though this may vary under adverse conditions (Lambers, 1987). Approximately 30% of the total ^{14}C partitioned to the roots was lost through root leakage and respired CO_2 in the 42/42 treatment, which could have been fixed by soil microorganisms. During the 6 hour treatment period, 3.5% of the total recovered ^{14}C was from the media fraction for the 42/42 treatment (Tables 2 and 3). The total ^{14}C found in or respired from both root halves for the 42/42 treatment was over three times greater than the ^{14}C found in the media for the 30/42 treatment.

In conclusion, 38 C appeared to be the upper threshold for root-zone temperature tolerance as indicated by a number of physiological and growth values in 'Rotundifolia' holly. Half of the root system exposed to 30 C can partially compensate in terms of shoot growth for detrimental effects to the root half at 34 and 38 C. If both root halves were grown at 38 C or just half of the root system was exposed to 42 C, decreased shoot growth occurred. Compensation is important because the temperature profile within a container will vary several degrees depending upon the orientation of the wall to incoming solar radiation (Martin and Ingram,

1988). Although total plant biomass varied between treatments, increasing root-zone temperatures did not appear to alter photosynthetic rates or root:shoot ratios. Therefore, while increasing root-zone temperatures affected overall plant growth, increasing root-zone temperatures did not affect sink strength so as to alter photosynthesis.

Foster (1986) proposed that decreases in the amount of ^{14}C found in the root system of 'Rotundifolia' holly when exposed to root-zone temperatures from 28 to 42 C could be due to a physical blockage of the vascular system or an increase in partitioning to the roots with subsequent loss of ^{14}C due to increased respiration rates. A physical blockage of the vascular system did not appear likely since no difference in the percent total recovered ^{14}C partitioned to the roots was found. However, the increased loss of respired ^{14}C at 38 C could account for the decrease in the labelled root fraction seen by Foster (1986). When both root halves were exposed to 42 C, the primary loss of current ^{14}C photosynthate most likely occurred through leakage due to loss of membrane integrity.

CHAPTER 3

THE EFFECT OF SUPRAOPTIMAL TEMPERATURES ON ROOT RESPIRATORY CHARACTERISTICS OF 'ROTUNDIFOLIA' HOLLY

Introduction

Increased root-zone temperatures are known to have profound effects on growth, assimilate partitioning and respiratory mechanisms of plants (Cooper, 1973). Root growth is often retarded by temperatures in excess of 30 C with cessation of top growth or necrosis occurring at temperatures of 40 C or above (Wong et al., 1971). Decreases in root dry weight (Barr and Pellett, 1972) and root growth (Johnson and Ingram, 1984) have been reported for several woody species exposed to root-zone temperatures above 40 C.

Temperatures as high as 45 C have been shown to increase respiration rates of actively growing tissues. Temperature can have significant effects on the maintenance costs of plant cells (Penning de Vries, 1975). Dark and maintenance respiration have been shown to each have a Q_{10} of 2 or more in a variety of plants as temperature increases (McCree, 1974, Hunt and Loomis, 1979). Respiration rate increases initially in response to supraoptimal root-zone temperatures, but decreases over time due to the progressive degradation of enzymatic processes (Langridge and McWilliam, 1967). Janes et al. (1988) found respiration rates in cultured tomato roots to be greatest at temperatures between 40 and 45 C after a 30 minute exposure which suggests that respiratory enzymes are functioning at supraoptimal temperatures.

Temperature differentially affects the electron transport pathways of plant mitochondria (Dizengremel and Chauveau, 1978). Two mitochondrial electron transport terminal oxidases are known, cytochrome *c* oxidase which is cyanide-sensitive (CN-sensitive), and the less

understood alternative oxidase which is CN-resistant. The CN-resistant pathway in plant mitochondria is considered to be an alternative, non-phosphorylating pathway which branches from the respiratory chain at ubiquinone (Henry and Nyns, 1975). Salicylhydroxamic acid (SHAM) is a substituted hydroxamic acid which specificly inhibits the CN-resistant pathway in isolated mitochondria and can be used to assess CN-resistant respiration (Schonbaum et al, 1971). Although concentrations of SHAM up to 25 mM can be used to estimate the activity of the CN-resistant pathway in plant tissue (Lambers et al, 1983), SHAM has been shown to stimulate O_2 consumption in roots (de Visser and Blaquiere, 1984; Spreen Brouwer et al., 1986). The stimulation of O_2 consumption at low SHAM concentrations (≤5 mM) is believed to be caused by a CN-sensitive peroxidase (Spreen Brouwer et al., 1986; van der Plas et al., 1987). The CN-resistant pathway is engaged under a variety of environmental stresses. In leaves of Fatsia japonica the CN-resistant respiratory pathway functioned between the temperatures of 6 to 30 C but declined significantly with temperatures up to 40 C (Burgos et al., 1987).

Working with Ilex crenata 'Rotundifolia', Foster (1986) found root respiration to increase after seven days with root-zone temperatures up to 40 C. Although total CO_2 respired from 'Rotundifolia' holly roots was not influenced by increasing root-zone temperature up to 42 C, increases in respiration of current photoassimilates at 38 C were detected (Chapter 2). Increased respiration can effect long-term plant growth and vigor in reaction to supraoptimal root-zone temperatures. These studies were conducted to determine: 1) the effect of high temperatures on respiratory rates of plants grown at ambient or supraoptimal root-zone temperatures, and 2) the effect of high temperature on the function of the CN-resistant and CN-sensitive respiratory pathways in 'Rotundifolia' holly.

Materials and Methods

Rooted stem-tip cuttings of Ilex crenata Thunb. 'Rotundifolia' were potted in 1200 ml clear plastic bags using 1000 cm^3 of Metro-Mix 300 (W.R. Grace and Co., Cambridge, MA). The

plants were grown in a glass greenhouse under natural daylength conditions for a minimum of 12 weeks before being transferred to a high light growth room three weeks before the initiation of each experiment.

The experiments were conducted in a 3.0 m by 7.6 m walk-in growth room with irradiance supplied by 18 1000-W phosphor coated metal-arc HID lamps (GTE Sylvania Corp., Manchester, NH). Photosynthetic photon flux density as measured with a quantum radiometer (LI-Cor Inc., Lincoln, NE) was 500 μmol s^{-1} m^{-2} at canopy height. The photoperiod was 13 hours daily (0700 to 2000 HR) with the dark period being interrupted for three hours (2200 to 0100 HR) with incandescent light. Air temperature was maintained at 28±1 C during the light period and 21±1 C during the dark period. Relative humidity was maintained at approximately 40% during the light period and 90% during the dark period. Plants were fertigated twice weekly with a 300 mg L^{-1} N solution (5.6% nitrate, 4.0% ammonium, 10.4% urea) of soluble 20N-8.8P-16.6K fertilizer (Peters 20-20-20, W.R. Grace and Co., Cambridge, MA).

Two experiments were designed to evaluate the effects of temperature on respiration in roots of 'Rotundifolia' holly. In experiment 1, the plants were grown at root-zone temperatures of 30, 34, 38 and 42 C for six hours daily for three weeks before respiration of excised roots was measured in buffer solution at the corresponding temperature or at 25 C. Also, plants were grown at a root-zone temperature of 30 C and respiration measurements were made at buffer solution temperatures of 25 to 70 C. For experiment 2, effects of temperature on the CN-resistant respiratory pathway and the CN-sensitive pathway were determined for the same treatments as in experiment 1 and from 25 to 54 C using different root tissue samples. For all experiments root-zone temperature treatments were imposed for 21 days in a completely randomized block design replicated a minimum of five times. Root-zone temperatures were maintained within ±1 C using an electronically controlled root-heating system previously described by Foster (1986).

Respiratory measurements were made with a Clark-type oxygen electrode (Hansatech, Kings Lynn, England). The temperature of the reaction vessel was maintained by circulating heated water through the water jacket. The buffer solution consisted of 0.3 M mannitol and 25mM Tes buffer adjusted to pH 7.1 with KOH plus appropriate concentrations of SHAM (0, 5, 10, and 15 mM) and KCN (0, 0.5 and 1.0 mM) when used. 100 mg samples of fibrous white roots approximately 1.0 cm in length were used. Root samples were pooled from a minimum of three plants and were kept moist until measurements were made. Rates of O_2 uptake were determined between minutes 8 and 10 after steady state uptake was achieved and were expressed on the basis of root fresh weight.

V_{res} is that portion of V_T (total respiration) which is insensitive to inhibitors of the CN-sensitive and CN-resistant pathways and is considered to be extra-mitochondrial in nature. The activity of the CN-resistant alternative pathway was determined using the method described by Lambers et al. (1983). Effects of SHAM on the CN-sensitive, cytochrome pathway were determined by reverse titration of roots with KCN in the presence and absence of SHAM (Theologis and Laties, 1978; de Visser and Blacquiere, 1984; Lambers and Day, 1987). Electrolyte leakage procedures for the determination of a critical temperature for 'Rotundifolia' holly roots were performed as described by Ingram and Buchanan (1981). Data was analyzed as a general linear model (Statistical Analysis System, Raleigh, NC) unless otherwise noted.

Results and Discussion

Effects of Temperature on Respiratory Rate

When the plants were grown at root-zone temperatures of 30 to 42 C and O_2 consumption was determined at 25 C, respiration rate decreased linearly with increasing root-zone growth temperature (R^2=0.98, V_T=0.189-0.0033(Temp)) (Figure 1). However, root-zone temperatures did not affect respiration rate when the buffer solution temperature was equal to the growth temperature. O_2 solubility decreases with increasing buffer solution temperature and could

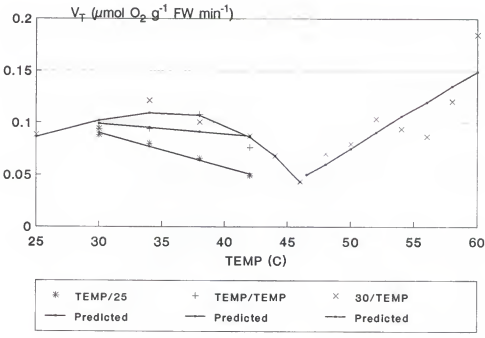

Figure 3-1. The influence of root-zone growth temperature and buffer solution temperature combinations on the respiratory rate (V_T) of Ilex crenata 'Rotundifolia' roots. Temp/25 (R^2=0.98) indicates the plants were grown at root-zone temperatures of 30, 34, 38 and 42 C and respiration was measured at 25 C. Temp/Temp (R^2=0.65) indicates the plants were grown and respiration measurements were made at the same temperature. 30/Temp indicates the plants were grown at a root-zone temperature of 30 C and respiration was measured at different temperatures (25 to 60 C). Respiration rates from 25 to 46 C responded quadratically (R^2=0.94) while rates from 46 to 60 responded linearly (R^2=0.76). Each value represents the mean of six independent determinations.

potentially limit respiration processes. These results showed that decreased respiration was due to root-zone growth temperature and not to decreased O_2 availability. Solution temperature up to 42 C did not adversely affect respiration rates due to decreasing O_2 levels in the buffer solution.

In Agave deserti, a succulent desert perennial, root respiration increased several fold from 5 to 40 C and decreased two-fold as temperature increased from 45 to 55 C (Palta and Nobel, 1989). Increased respiration rates up to 45 C have generally been attributed to increased maintenance respiration, and/or changes in enzyme activation energy relative to changes in temperature. Variation in respiration rates at different buffer solution temperatures (Temp/Temp at 38 and 42 C versus Temp/25 at 38 and 42 C) in experiment 1 (Figure 1) may be due to increased maintenance respiration costs when the roots were exposed to higher buffer solution temperatures. Maintenance respiration increases with increasing temperature to produce energy for maintaining cellular structure, ion gradients and for making physiological adaptations necessary to maintain cellular integrity in changing environments.

Enzyme catalyzed reactions are sensitive to small changes in temperature in the biologically active range. Many enzymes are labile in the range of 40 to 50 C. Above an optimum temperature, catalytic effectiveness decreases due to enzyme inactivation or denaturation. Root respiration in 'Rotundifolia' holly increased when plants were exposed to temperatures up to 40 C for one week (Foster, 1986). The decrease in respiration seen in this study as root-zone temperature increased from 30 to 42 C when measured at 25 C may be explained by the loss of catalytic effectiveness of certain respiratory enzymes after repeated, prolonged exposure to elevated temperatures. The elevated rates seen at growth temperatures of 38 and 42 C when the buffer solution was also 38 and 42 C, respectively, could then be due to decreased activation energy with increasing temperature.

Two different responses were seen as roots grown at 30 C were exposed for 10 minutes to buffer solution temperatures from 25 to 60 C (Figure 1). A quadratic response described the

relationship of respiration to temperature in the range of 25 to 46 C ($Pr>F=0.01$, $R^2=0.94$, $V_T=0.3559-0.0349(Temp)+0.0014(Temp)^2$). Maximum O_2 consumption occurred at 34 C (0.121 μmol min^{-1}/gFW) while respiration decreased by approximately 50% at 46 C in relation to the rate at 25 C. O_2 consumption increased linearly with temperature from 46 to 60 C ($Pr>F=0.01$, $R^2=0.76$, $V_T=-0.2965+0.0074(Temp)$). At 65 and 70 C, O_2 consumption (0.057 and 0.032 μmol min^{-1}/gFW, respectively) decreased below the control value (0.088 μmol min^{-1}/gFW).

O_2 consumption in the range of 25 to 46 C was probably due to mitochondrial respiratory processes, while increased O_2 consumption at temperatures above 46 C may be due to lipid oxidation caused by peroxidases (Levitt, 1980). Peroxidases are extremely heat stable enzymes which have been implicated in the SHAM stimulation of O_2 uptake in intact roots of several species (de Visser and Blacquiere, 1984; Spreen Brouwer et al., 1986). The lethal temperature of 'Rotundifolia' holly roots was predicted to be 48±1.5 C for a 30 minute exposure as determined by electrolyte leakage procedures (Figure 2). This lethal temperature corresponds with the temperature initiating an increase in O_2 consumption (46 to 48 C). Baaziz (1989) found that a 20 minute exposure to 60 C did not completely inactivate all peroxidase isozymes from date palm leaves. In preliminary studies, 'Rotundifolia' holly roots exposed to 44 to 60 C for 10 minutes in buffer solution, continued O_2 consumption after the roots were removed. In barley roots, peroxidase was easily washed from the roots, but was not active unless exogenous NADH was added (Bingham and Farrar, 1987). Since an apparent loss of membrane integrity occurs in 'Rotundifolia' holly near 48 C, it was quite reasonable that reductants such as NADH necessary for the functioning of a peroxidase may have leaked out into the buffer solution at temperatures above 46 C.

Effect of Temperature on the CN-sensitive and CN-resistant Pathways

The effect of inhibitor concentrations on root respiration was determined for plants grown at a root-zone temperature of 30 C using a buffer solution temperature of 25 C. Maximum inhibition of respiration (66% decrease) occurred with 1.0mM KCN and 15.0 mM SHAM. SHAM

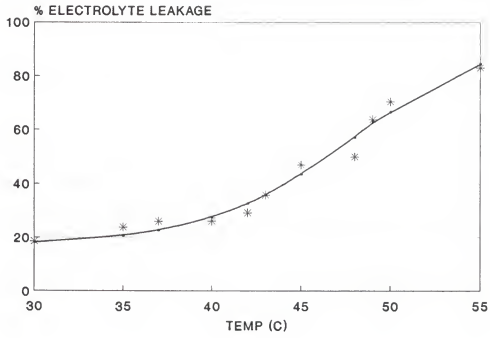

Figure 3-2. Electrolyte leakage from excised roots of 'Rotundifolia' holly after a 30 minute exposure to temperatures in the range of 30 to 55 C. The critical temperature (48.0±1.5 C) was determined from a fitted sigmoidal curve as described by Ingram and Buchanan (1984). Each point is the mean of eight replications.

alone (15.0 mM) decreased respiration (V_T) by approximately 40% while residual respiration (V_{res}) accounted for 33% of total respiration. The inhibition caused by SHAM indicates the CN-resistant pathway was engaged in vivo in holly roots (Moller et al., 1988). Potassium cyanide, the inhibitor of the CN-sensitive, cytochrome pathway, decreased respiration by approximately 22% at a concentration of 1.0 mM. The effects of SHAM on the CN-sensitive, cytochrome pathway were examined by reverse titration of roots with KCN in the presence and absence of SHAM according to Theologis and Laties (1978) and de Visser and Blaquiere (1984). The resulting p values (slope of the resulting regression relationship) for KCN at 0.5 and 1.0 mM were 1.48 and 1.05, respectively. According to de Visser and Blacquiere (1984), a p value greater than 1 indicates that SHAM inhibits the flow of electrons through the CN-sensitive, cytochrome pathway. A value of 1 indicates that SHAM had no effect on the cytochrome pathway. Accordingly, the concentrations of SHAM used in this study could be used in conjunction with 1.0 mM KCN to inhibit the cyanide-resistant pathway in 'Rotundifolia' holly roots since p at 1.0 mM KCN was not different from 1.

Effects of root-zone growth temperature and increasing buffer solution temperature on the CN-sensitive and CN-resistant pathways are presented in Table 1. Percent SHAM inhibition ranged from a low of 26.7% for the 30-46 (root-zone growth temperature - buffer solution temperature) treatment to a high of 58.7% for the 30-25 treatment. That portion of respiration resistant to KCN (%CN-resistance) ranged from a high of 93.3% (30-46) to a low of 53.9% (42-42). Lambers et al. (1983) reported a range of 0 to 44% SHAM inhibition and 32 to 64% CN-resistance for intact roots of several agronomic annual species. Residual respiration (V_{res}) accounted for up to 40% of total respiration in crowns of frost-sensitive and frost-tolerant winter wheat cultivars (Rybka, 1989). Lambers and Day (1987) noted that residual respiration rarely exceeds 10% in roots. In our study, residual respiration ranged from a low of 27.9% (34-25) to a high of 70.4% (30-46). This high degree of residual respiration indicates the presence of extra-mitochondrial oxidases which are resistant to both SHAM and KCN or poor penetration

Table 3-1. Effects of root-zone temperature and increasing buffer solution temperature on the CN-sensitive and CN-resistant respiratory pathways. Activity of CN-resistant respiration (P') was calculated as the ratio of (SHAM inhibition)/(CN-resistance). Values presented are the means±SE of at least five independent determinations. The concentrations of effectors used were: SHAM (15mM) and KCN (1.0mM).

Growth°C	Buffer°C	%SHAM Inhibition	%CN-resistance	P'	V_{res}
30	30	35.7 (10.9)	57.0 (2.4)	0.63 (0.21)	39.8 (2.4)
34	34	34.0 (12.6)	58.7 (11.2)	0.58 (0.22)	35.6 (8.5)
38	38	28.7 (9.8)	60.2 (13.0)	0.48 (0.20)	39.5 (4.5)
42	42	35.5 (6.1)	53.9 (6.1)	0.66 (0.20)	42.9 (4.5)
30	25	58.7 (6.1)	59.0 (8.3)	0.99 (0.19)	34.3 (8.9)
34	25	27.4 (5.2)	41.3 (2.1)	0.67 (0.12)	27.9 (5.6)
38	25	39.4 (9.2)	62.1 (8.1)	0.63 (0.14)	44.0 (8.9)
42	25	38.8 (8.5)	55.1 (8.8)	0.70 (0.32)	33.8 (4.0)
30	25	58.7 (6.1)	59.0 (8.3)	0.99 (0.19)	34.3 (8.9)
30	30	35.7 (10.9)	57.0 (2.4)	0.63 (0.21)	39.8 (2.4)
30	34	50.4 (7.2)	61.4 (8.3)	0.82 (0.22)	41.6 (3.4)
30	38	37.5 (5.2)	59.6 (6.7)	0.63 (0.17)	48.0 (6.5)
30	42	38.5 (11.1)	66.1 (8.3)	0.69 (0.23)	53.2 (5.1)
30	46	26.7 (11.4)	93.3 (6.4)	0.29 (0.12)	70.4 (6.4)
30	50	42.3 (5.0)	63.9 (8.8)	0.66 (0.16)	40.5 (5.8)
30	54	39.0 (5.8)	79.0 (7.7)	0.49 (0.20)	52.3 (7.8)

of inhibitors into the roots. Large residual respiration values in certain tissues are unlikely to be caused by poor penetration of inhibitors (Lambers, 1987). However, greater suberization in 'Rotundifolia' holly root segments compared to succulent root tips used in other studies may have decreased inhibitor penetration which may have accounted for some of the variation seen in this study (Table 1). No increase in O_2 consumption was seen at low concentrations of SHAM for intact holly roots in our study, thus indicating that peroxidases were not stimulated.

The activity of CN-resistant respiration (P') was calculated from the ratio of SHAM inhibition divided by CN-resistance (Lambers et al., 1983a). Cyanide-resistant respiration was fully activated (P'=0.99) in plants grown at a root-zone temperature of 30 C with respiration measured in buffer solution at 25 C. No differences in CN-resistant pathway activity were detected when root growth temperature was the same as buffer solution temperature. When the buffer solution was maintained at 25 C, the 34 and 38 root-zone temperature treatments exhibited decreased CN-resistant pathway activity compared to the control (30 C).

In leaves of Fatsia japonica, the capacity of the CN-resistant pathway was high between 6 and 30 C (0.81 to 0.98) and declined significantly at 36 and 40 C (Burgos et al., 1987). The activity of the CN-resistant pathway was fully engaged at 25 C, ranged from 63 to 82% activation between 30 to 42 C and showed a decrease to 29% activation at 46 C when roots were grown at 30 C and exposed to increasing buffer solution temperatures. Several authors have proposed that decreased O_2 availability at high temperatures due to decreased O_2 solubility may explain why the alternative pathway accounts for a greater portion of total respiration at low temperatures compared to higher temperatures (Cook and Cammack, 1985; McCaig and Hill, 1977; Purvis, 1988). Temperature did not have a significant effect on the activity of the CN-resistant pathway as buffer solution temperature increased until the temperature reached 46 C, indicating that decreased O_2 solubility with increasing temperature did not limit respiratory functions during this short exposure period. The decrease in CN-resistant pathway activity at 46 C corresponds to a decrease in SHAM inhibition and an

increase in CN-resistance (93.3%). These differences may also be due to thermal effects on the oxidases themselves or temperature-induced alterations in membrane structure.

Oxidative phosphorylation was found to be rapidly destroyed by 40 C for 60 minutes in mitochondria from cauliflower and potato (Dizengremel and Chauveau, 1978). Nakagawa et al. (1988) found cytochrome *c* oxidase in above ground organs of different species to be more resistant to heat inactivation than that found in below ground organs after 60 minute exposures of 40 to 55 C. Like cytochrome *c* oxidase and other electron transport system components, the alternative oxidase is located in the inner mitochondrial membrane. The loss of CN-resistant activity at 46 C corresponded to the temperature (48±1.5 C) disrupting holly root membranes, thus leading to the disorganization of the electron transport system. The "recovery" of activity at 50 C was attributed to an uncharacterized oxidase which appeared to be stimulated by temperatures above 46 C.

It was proposed that the CN-resistant pathway served a protective role against temperature extremes, functioning in reserve in case of failure of the more sensitive CN-sensitive pathway (Kiener and Bramlage, 1981). Since no difference occurred for CN-resistant pathway activity in the range of 25 to 42 C, it was concluded that engagement of the CN-resistant pathway was not dependant upon exposure to temperature extremes. Lambers (1982) suggested that the CN-resistant pathway functions as an "energy overflow" which removes excess carbohydrates when substrate levels in the cell are high and the need for carbon skeletons and ATP is low. de Visser et al. (1986) proposed the concept of the CN-resistant pathway acting as an "overcharge overflow" where under circumstances of increased cellular need for ATP, the CN-resistant pathway would be engaged as an inefficient method for supplementing ATP produced through the cytochrome pathway. Further research would be required before the views of Lambers (1982) or de Visser et al. (1986) could be distinguished relative to the respiratory response of the CN-resistant pathway to increasing root-zone temperature in 'Rotundifolia' holly.

CHAPTER 4

INFLUENCE OF SUPRAOPTIMAL ROOT-ZONE TEMPERATURES ON PHOTOSYNTHETIC MECHANISMS IN 'ROTUNDIFOLIA' HOLLY

Introduction

Photosynthesis is controlled by a variety of environmental and biochemical factors. One environmental factor that influences photosynthetic parameters is high temperature stress. Among the factors involved in the loss of photosynthetic activity at high temperatures are non-stomatal limitations (Bauer, 1978; Zioni and Itai, 1972) which include ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) activity (Al-Khatib and Paulsen, 1984; Monson et al., 1982), and changes in chlorophyll fluorescence associated with damage to photosystem II (PSII) activity (Smillie and Nott, 1979; Bilger et al., 1984; Havaux et al., 1988).

RuBisCO activity is influenced by high temperatures and has been positively correlated with photosynthetic activity (Downton and Slayter, 1972; Pearcy, 1977). RuBisCO activity decreased over time in response to supraoptimal leaf temperatures in wheat (Al-Khatib and Paulsen, 1984). Badger et al. (1982) found the activity of RuBisCO in oleander plants grown at 20 C was approximately 1.5 times greater than plants grown at 45 C. In ivy leaves, RuBisCO was found to be heat stable with little loss in activity until leaf tissue was lethally damaged at 52 C (Bauer and Senser, 1979).

Although stomatal limitations of photosynthesis in response to high temperature have been reported, there have also been reports of non-stomatal inhibition (Bar-Tsur et al., 1985; Bauer, 1978; Monson et al., 1982; Pearcy, 1977). In many instances, stomatal limitations of CO_2 assimilation decreased in response to environmental stresses (Farquhar and Sharkey, 1982).

For C_3 plants, non-stomatal limitations of photosynthesis caused by environmental stresses can be analyzed by comparing the relationship between CO_2 assimilation rate (A) and leaf intercellular CO_2 concentration (c_i) when RuBP is considered to be saturating (low C_i) or limiting (high c_i). Little information is known about relationships between CO_2 assimilation rates and internal CO_2 concentrations in woody plants (Davis et al., 1987), particularly in regards to environmental stresses.

At room temperature, light excites chlorophyll which causes fluorescence to be emitted from chlorophyll a of photosystem II (PSII) in the wavelength range of 680 to 720 nm. PSII and the water-splitting complex have been found to be extremely sensitive to high temperature stress (Berry and Bjorkman, 1980). Chlorophyll fluorescence was found to be a simple method for studying the photosynthetic response of plants to environmental stresses (Smillie and Hetherington, 1983; Havaux et al., 1988). Non-variable or initial fluorescence (F_o), the fluorescence level which occurs when all the reaction centers of PSII are open, increased when plant leaves were heat stressed (Schreiber and Berry, 1977; Smillie and Nott, 1979).

While considerable research has been conducted on photosynthetic responses of above-ground plant parts to high temperature stress, little is known about the response of photosynthetic mechanisms to supraoptimal root-zone temperatures. Decreases in photosynthetic rates of woody plants in response to supraoptimal root-zone temperatures have been reported (Gur et al., 1972; Johnson and Ingram, 1984). Foster (1986) reported a decrease in shoot carbon exchange rate which was determined to be non-stomatal when 'Rotundifolia' holly plants were grown at root-zone temperatures of 36 and 40 C compared to 28 and 32 C for one week. Therefore, the objectives of this research were to determine the effects of root-zone temperature on RuBisCO activity, net CO_2 assimilation (A), internal CO_2 levels (c_i), stomatal conductance (C_s), transpiration (E), fluorescence kinetics, pigment levels and A-c_i response curve relationships.

Materials and Methods

Plant Material and Growth Conditions

Rooted stem-tip cuttings of Ilex crenata Thunb. 'Rotundifolia' were potted into 1200 ml clear plastic bags using 1000 cm^3 Metro-Mix 300 (W.R. Grace and Co., Cambridge, MA) as a growth medium. Plants were grown in a glass greenhouse under natural daylength conditions for a minimum of 12 weeks before being transferred to a high-light growth room three weeks before the initiation of experiments.

Experiments were conducted in a 3.0 m by 3.6 m walk-in growth room with irradiance supplied by 12 1000-W phosphor-coated, metal-arc HID lamps (GTE Sylvania Corp., Manchester NH). Photosynthetic photon flux density was 850±50 μmol s^{-1} m^{-2} at canopy height measured with a quantum radiometer (Li-Cor Inc., Lincoln, NE). The photoperiod was 13 hours daily (0800 to 2100 HR) with the dark period being interrupted for three hours with incandescent light. Air temperatures and relative humidity, respectively, were maintained at 28±1 C and 40% during the light period and 21±1 C and 90% during the dark period. Plants were fertigated twice weekly with a 300 mg L^{-1} N solution of soluble 20N-8.8P-16.6K fertilizer (Peters 20-20-20, W.R. Grace and Co., Cambridge, MA).

Root-zone temperature treatments of 30, 34, 38 and 42 C were maintained within ± 1 C for 6 hours daily for 21 days for all experiments using an electronically controlled root-heating system (Foster, 1986). All data were analyzed as general linear models unless otherwise stated.

Extraction and Assay of RuBisCO Activity

The extraction and assay of HCO_3^-/Mg^{2+} - activated (total) RuBisCO activity (carboxylase activity of the enzyme) from leaf samples was determined using methods modified from Lorimer et al. (1976). Five replicate plants were used. Leaf samples were collected approximately 2 hours into the light period and were frozen at -80 C. One gram fresh weight samples were ground and homogenized in 5 ml ice-cold extraction buffer consisting of 100 mM Tris-HCl (pH

8.0), 10 mM $MgCl_2$, 5 mM dithiothreitol (DTT), 1.5% w/v PVP-40 and 10 mM D-Isoascorbic acid. Samples were centrifuged at 9500 g for 6 minutes at 4 C. Assays were performed at 25 C using sample vials which contained 400 μl assay medium and control vials which contained 425 μl. Control assays differed in that RuBP was not added. All vials were capped with rubber septa and sealed with masking tape. The assay medium contained 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 10 mM D-Isoascorbic acid and 5.0 mM DTT. Syringes were used to add reactants to each vial. Once 20 μl of 0.5 M $NaH^{14}CO_3$ (2 μCi) were added to each vial, 50 μl of crude leaf enzyme extract were added to all vials and samples were pre-incubated for 5 minutes. Assays were initiated by the addition of 25 μl of 5.0 mM RuBP for one minute and terminated by the addition of 50 μl of 6 N HCl. Controls were terminated after preincubation because RuBP was not added. Samples were uncapped and allowed to dry in a fume hood for 48 hours. The incorporation of acid-stable ^{14}C was measured by liquid scintillation spectrometry after the addition of 400 μl distilled H_2O and 4 ml scintillation fluid (ACS-II, Amersham Corp., Arlington Heights, IL) to each vial. Total soluble protein was determined from extract supernatants according to the method of Bradford (1976). Total chlorophyll was determined according to the methods of Bruinsma (1963), measuring absorbance at 652 nm.

Measurement of CO_2 Response Curves

Photosynthetic CO_2 assimilation rate versus internal CO_2 concentration relationships ($A\text{-}c_i$) were measured with a Li-Cor portable photosynthesis system consisting of a LI-6200 computer and a LI-6250 gas analyzer (Li-Cor Inc., Lincoln, NE). Canopy gas exchange measurements were made using a custom-designed 7.1 L plexiglass chamber. Root-zone temperatures were maintained electronically as previously described by Foster (1986). A high pressure sodium vapor lamp (Lumalux 400W, GTE Sylvania Inc., Manchester, NH) was used to provide a photosynthetic photon flux density of 1100±25 μmol s^{-1} m^{-2} at canopy height. Initial CO_2 levels were elevated to approximately 900 μl CO_2 L^{-1} by breathing into the chamber. CO_2 drawdown (50 μl CO_2 L^{-1} increments) in the range of 800 to 150 μl CO_2 L^{-1} was measured by using the

LI-6200 in closed mode and allowing the plant canopy to decrease the CO_2 concentration (Davis et al., 1987). Gas exchange values were replicated three times in increments of five seconds in the range of 450 to 800 μl CO_2 L^{-1}, four seconds from 200 to 400 μl CO_2 L^{-1}, and three seconds for 150 μl CO_2 L^{-1}. For each time change, the chamber was opened for 5 minutes to alleviate problems associated with increasing relative humidity above 70%. Maximum flow rates of equipment were not sufficient to adequately maintain relative humidity during measurement periods required below 150 μl CO_2 L^{-1}.

For each measurement made at a given CO_2 level, temperature was maintained within $\pm$0.1 C. Air temperature in the canopy was maintained at 29 $\pm$1 C during the course of the experiment. Measurements for each plant took approximately 45 minutes. After gas exchange measurements were completed, leaf area was determined from a prediction equation using leaf fresh weight (R^2=0.99, leaf area=5.20(leaf fresh weight) + 39.37). Assimilation (A= μmol CO_2 s^{-1} m^{-2}), internal CO_2 (c_i= μl CO_2 L^{-1}), stomatal conductance (C_s= cm s^{-1}) and transpiration (E= mol m^{-2} s^{-1}) were calculated in accordance with the equations of von Caemmerer and Farquhar (1981). Percent stomatal limitation (L) was calculated according to Farquhar and Sharkey (1982).

Fluorescence Measurements

The youngest fully-expanded leaf was selected from each of eight replicate plants from root-zone temperature treatments, placed on moist filter paper in a petri dish and dark acclimated for 60 minutes. Each leaf was then placed in an environmentally controlled leaf chamber (Hansatech LD2, Hansatech, Kings Lynn, England). Leaf chamber temperature was maintained at 25 C using a Lauda RM6 (Brinkman Instruments Co., Westbury, NY) circulating water bath. CO_2 levels were maintained in the leaf chamber by using a 1 M solution of sodium bicarbonate. A template was constructed which allowed a 50 mm^2 area of leaf tissue to be exposed to 660 nm light for photosynthesis. Chlorophyll *a* fluorescence was detected using a Hansatech fluorescence detector fitted with a 740 nm interference filter. Data were recorded

every 6.9 milliseconds with a LeCroy 8210 waveform analyzer transient recorder (LeCroy Corp., Spring Valley, NY) relayed through a LeCroy 8901A GPIB interface connected to an IBM PC for data storage. From the data, F_o (initial fluorescence), F_m (maximal fluorescence), F_v (variable fluorescence = F_m-F_o), F_v/F_m (estimated quantum yield) and q_Q (photochemical quenching = $(F_m-F_v)/(F_m-F_o)$) were calculated. Total chlorophyll, chlorophyll *a* and *b* and total carotenoids were determined according to the methods of Bruinsma (1963). Discs from leaves on which fluorescence measurements were made were extracted in 80% v/v aqueous acetone for 24 hours in the dark at 4 C before analysis. An analysis of covariance was used for fluorescence and pigment data using pretreatment measurements as the covariate to account for pretreatment differences.

Results and Discussion

CO_2 assimilation can be limited by changes in stomatal conductance and/or the capacity of mesophyll tissue to perform photosynthetic processes. The photosynthetic activity in the mesophyll is dependant upon the activity of RuBisCO, and the capacity of photosynthetic electron transport to regenerate RuBP. Environmental effects on photosynthetic parameters can be determined by assessing the relationship between net CO_2 assimilation (A) and internal CO_2 concentrations (c_i) (von Caemmerer and Farquhar, 1981; Farquhar and Sharkey, 1982). The advantage of this procedure is that the influence of environmental effects on photosynthesis can be determined independent of stomatal effects. The relationship between A and c_i for 'Rotundifolia' holly was significantly quadratic ($Pr>F=0.01$, $R^2=0.99$) for each root-zone temperature treatment (Figure 1).

CO_2 assimilation rate at low c_i concentrations, where RuBP levels are saturating and net CO_2 assimilation is dependant upon the partial pressure of CO_2 at sites of carboxylation (von Caemmerer and Farquhar, 1981), is described by a linear demand function . Since the partial pressure of CO_2 at the site of carboxylation is assumed to be equal to c_i, the linear portion of

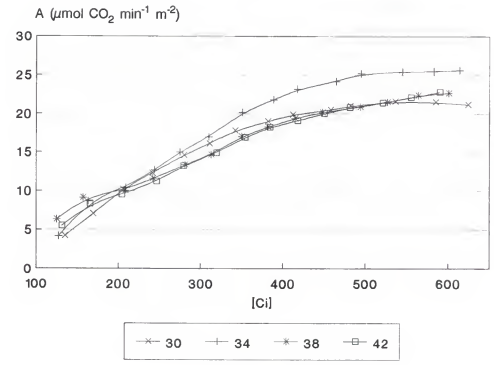

Figure 4-1. The relationship between CO_2 assimilation rate (A) and internal CO_2 levels (c_i) for 'Rotundifolia' holly after exposure for 21 days to root-zone temperatures of 30, 34, 38 and 42 C. Each value is the mean of four replicate plants. The following equations describe the A-c_i relationship for each root-zone temperature: 30 C:(R^2=0.99, c_i=51.31 + 0.568(CO_2) + 0.0001(CO_2)^2), 34 C: (R^2=0.99, c_i=34.82 + 0.658(CO_2) + 0.00009(CO_2)^2), 38 C:(R^2=0.99, c_i=19.98 + 0.729 (CO_2) + 0.00001(CO_2)^2), 42 C:(R^2=0.99, c_i=16.01 + 0.777(CO_2) + 0.00001(CO_2)^2).

the A-c_i curve (Figure 2) is proportional to the maximal activity of RuBisCO in the leaf. At root-zone temperatures of 30 and 34 C, the slopes of the A-c_i relationships in the range of 125 to 250 μL CO2 L^{-1} were 0.078 and 0.074, respectively. The initial A-c_i slopes at 38 and 42 C were significantly decreased (0.044 and 0.047, respectively) compared to the 30 and 34 C root-zone temperature treatments, which indicated greater RuBisCO activity at the lower root-zone temperatures. If the slope of A-c_i line in the linear phase was proportional to the activity of RuBisCO, then RuBisCO activity in this study was approximately 65% greater in plants grown with root-zone temperatures of 30 and 34 C compared to 38 and 42 C (Figure 2).

RuBisCO activity in response to increasing root-zone temperature is shown in Table 1. RuBisCO comprises approximately 20% of the soluble protein in leaves (Sharkey, 1985). Soluble protein levels increased with increasing root-zone temperature. RuBisCO activity per unit protein responded linearly (R^2=0.71) to increasing root-zone temperature. Vu and Yelenosky (1987) found that soluble protein levels decreased in 'Valencia' orange leaves after shoots were exposed to high temperature (32.2 C) treatments.

RuBisCO activity per unit fresh weight also increased with increasing root-zone temperature (Table 1). It is possible that RuBisCO activation state decreased with increasing root-zone temperature. The increase in soluble protein with increased root-zone temperature may actually have been an increase in RuBisCO protein levels. If RuBisCO activation state was decreased, then increased levels of RuBisCO protein would be required to maintain CO_2 assimilation. Increased root-zone temperatures were shown to affect RuBisCO activation state in response to altered root sink strength (Hurewitz and Janes, 1987). Exposure of 'Rotundifolia' holly roots to 28, 34 and 40 C for six weeks linearly decreased nitrogen levels in roots and shoots with increasing root-zone temperature (Harrison, 1989). Since RuBisCO is a major nitrogen sink in leaves, it is possible that 'Rotundifolia' holly was able to reallocate nitrogen to the formation of RuBisCO to maintain RuBisCO activity for photosynthesis. Further studies on RuBisCO activation state and RuBisCO protein quantity would be necessary to address these possibilities.

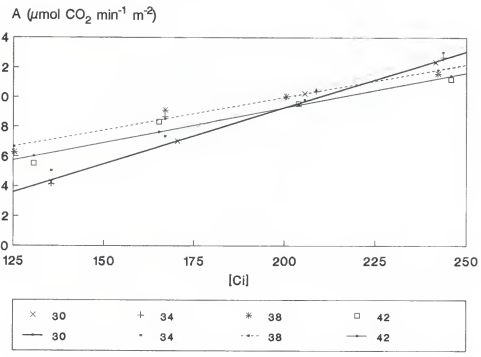

Figure 4-2. The linear phase relationship between CO_2 assimilation rate (A) and internal CO_2 levels (c_i) for 'Rotundifolia' holly after exposure for 21 days to root-zone temperatures of 30, 34, 38 and 42 C. The top row of symbols in the legend are means of four replicate plants. The bottom row in the legend represents the following regression equations for each root-zone temperature: 30 C:(R^2=0.99, -6.21 + 0.078(c_i)), 34 C:(R^2=0.93, -4.96 + 0.074(c_i)), 38 C:(R^2=0.96, 1.12 + 0.044(c_i)), and 42 C:(R^2=0.96, -0.124 + 0.047(c_i)).

Table 4-1. Effect of increasing root-zone growth temperature on RuBisCO activity in leaves of 'Rotundifolia' holly after 21 days of treatment at 30, 34, 38 and 42 C. Values presented are the means±SE of five replicate plants.

ROOT-ZONE TEMP C	SOLUBLE PROTEIN (mg g^{-1} FW)	RuBisCO ACTIVITY (μmol CO_2 hr^{-1} mg^{-1} protein)	RuBisCO ACTIVITY (μmol CO_2 hr^{-1} g^{-1} FW)	RuBisCO ACTIVITY (μmol CO_2 hr^{-1} μg^{-1} Chl)
30	13.64 (1.14)	0.572 (0.14)	8.03 (1.27)	0.095 (0.05)
34	13.02 (1.06)	0.801 (0.19)	9.94 (0.65)	0.220 (0.11)
38	16.52 (1.06)	0.636 (0.14)	10.35 (1.55)	0.141 (0.03)
42	18.62 (1.14)	0.580 (0.08)	10.24 (0.67)	0.175 (0.08)
Significance[z]	L*	L*	L**	L**

[z]Linear (L) or nonsignificant (ns) at the 5% (*) or 1% (**) level.

RuBisCO activity per unit chlorophyll apparently increased linearly (R^2=0.68) with increased root-zone temperature (Table 1), although the change in RuBisCO activity may have been due to decreased chlorophyll levels. Total chlorophyll levels decreased linearly (Pr>F=0.03, R^2=0.93) with increased root-zone temperature (data not shown). Chlorophyll *a* and chlorophyll *b* levels decreased linearly with increased root-zone temperature (Figure 3). Chlorophyll *a* decreased at a greater rate than chlorophyll *b* as root-zone temperature increased. As a result, the chlorophyll *a*/*b* ratios at 38 and 42 C (2.91 and 2.97±0.42, respectively) were significantly different from plants grown at 30 C (4.33±0.41). Total carotenoids also decreased linearly with increased root-zone temperature (Figure 4).

Root-zone growth temperature had no significant effect on fluorescence parameters after one week (data not shown) or after three weeks (Table 2). No increase in F_o, a common response in heat-stressed leaves (Havaux et al., 1988), was found in this study. F_v/F_m estimates the quantum yield of PSII primary photochemistry (the reduction of Q_A). The values for F_v/F_m in this study (0.787 to 0.801±0.014) are slightly lower than other values (0.83) reported for C_3 species (Bjorkman and Demmig, 1987). Photochemical quenching (q_Q), the fluorescence quenching due to the oxidation of Q_A, ranged from 0.249 (34 C) to 0.270 (30 C) with no differences due to treatment. The absence of differences in fluorescence parameters in relation to increased root-zone temperatures indicated that changes in CO_2 assimilation rate were not associated with damage to the photosynthetic electron transport system. Differences in photosynthetic parameters may have been due to factors associated with decreased pigment levels. Fewer light harvesting pigments would decrease the interception of quanta, thereby decreasing the number of electrons transported for the generation of NADPH and ATP necessary for carbon metabolism.

Stomata control the diffusive resistance of water vapor and CO_2 between the leaf and ambient air. Stomata respond to c_i (Mott, 1988) and, therefore, have the capacity to limit carbon fixation when c_i is not saturating. Graves et al. (1989) reported increased leaf diffusive

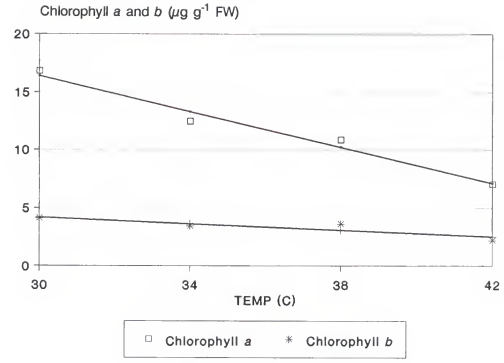

Figure 4-3. Chlorophyll *a* and *b* levels ($\mu g\ g^{-1}$ FW) for leaves of 'Rotundifolia' holly after 21 days at root-zone temperatures of 30, 34, 38 and 42 C. Each value is the adjusted mean from analysis of covariance using pretreatment values as the covariate. The relationship between chlorophyll *a* and root-zone temperature was described as (R^2=0.97, Chl *a*=39.64-0.774(Temp)). The relationship between chlorophyll *b* and temperature was described as (R^2=0.78, Chl *b*=8.360-0.139(Temp)).

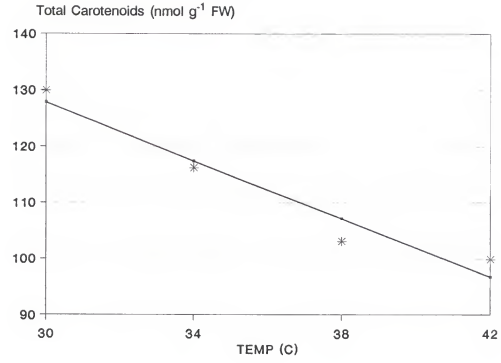

Figure 4-4. Total carotenoid levels (nmol g^{-1} FW) from leaves of 'Rotundifolia' holly after 21 days at root-zone temperatures of 30, 34, 38 and 42 C. Each value is the adjusted mean from analysis of covariance using pretreatment values as the covariate. The relationship between total carotenoids and temperature was described as (R^2=0.94, total carotenoids=205.37-2.58(Temp)).

Table 4-2. Chlorophyll a fluorescence for Ilex crenata 'Rotundifolia' exposed to root-zone temperatures of 30, 34, 38 and 42 C for 21 days. F_o = initial fluorescence, F_m = maximal fluorescence and F_v = variable fluorescence are expressed as relative values. Estimated quantum yield was = (F_v/F_m) and photochemical quenching = (q_Q). One leaf from eight replicate plants at each root-zone temperature was dark acclimated for 60 minutes before fluorescence measurements were made.

Root-zone Temperature C	F_o	F_m	F_v	F_v/F_m	q_Q
30	0.90 (0.06)	4.41 (0.20)	3.47 (0.17)	0.789 (0.014)	0.27 (0.02)
34	0.77 (0.06)	3.85 (0.19)	3.06 (0.16)	0.801 (0.014)	0.25 (0.02)
38	0.79 (0.06)	3.91 (0.19)	3.10 (0.16)	0.787 (0.014)	0.27 (0.02)
42	0.84 (0.06)	3.90 (0.20)	3.13 (0.17)	0.797 (0.014)	0.26 (0.02)
Significance[z]	L^{ns}	L^{ns}	L^{ns}	L^{ns}	L^{ns}

[z]Linear (L) or nonsignificant (ns) at 5% level (*).

resistance in Acer rubrum after a five-week exposure to a continuous root-zone temperature of 36 C. Newman and Davies (1988) noted decreased transpiration in Pittosporum tobira Thunb. 'Wheeler' at root-zone temperatures above 25 C. Foster (1986) attributed decreased rates of CO_2 assimilation in 'Rotundifolia' holly with increasing root-zone temperature to non-stomatal limitations based on the premise that transpiration increased after heating of roots. Stomatal conductance (Cs) and transpiration (E) decreased linearly with increasing CO_2 levels (Figures 5 and 6). Stomatal conductance decreased with increasing CO_2 to a greater extent at 42 C compared to the other root-zone temperature treatments. At 38 C, the rate at which E decreased with increasing CO_2 was greater compared to other root-zone temperature treatments. At 350 μL CO_2 L^{-1}, E decreased 11% at 42 C compared to the other three treatments (data not shown). However, at 800 μL CO2 L^{-1}, E for root-zone treatments of 38 and 42 C was significantly less than for the 30 and 34 C treatments. Therefore, the decreases in E at 350 and 800 μL CO_2 L^{-1} associated with the change in C_s at 42 C over the entire range of CO_2 levels may represent a dehydration prevention mechanism minimizing dehydration in response to supraoptimal root-zone temperatures. Stomatal conductance and transpiration were decreased in banana and Ixora after exposure to root-zone temperatures of 43 C (Ramcharan, 1987). Despite differences in C_s and E for the 38 and 42 C treatments, plants were able to maintain CO_2 assimilation at rates which did not differ from the control. Comparison of CO_2 assimilation rates at 350 μL CO_2 L^{-1} showed that they were significantly greater at 34 C (15.88) compared to 30, 38 and 42 C (14.55, 14.56, and 14.02, respectively). Maximum midday CO_2 assimilation occurred at a root-zone temperature of 33 C in banana and Ixora (Ramcharan, 1987). Percent stomatal limitation (L) at 350 μl CO_2 L^{-1} was significantly less at 34 C (-9.2%) compared to the other three treatments. Calculated L at 38 and 42 was not different from the control. The same response for L was seen at 800 μl CO_2 L^{-1}. No difference in L at 38 and 42 C was seen when compared to 30 C after exposure for three weeks. Thus, the increase in CO_2 assimilation at a root-zone temperature of 34 C was attributed to decreased stomatal limitations.

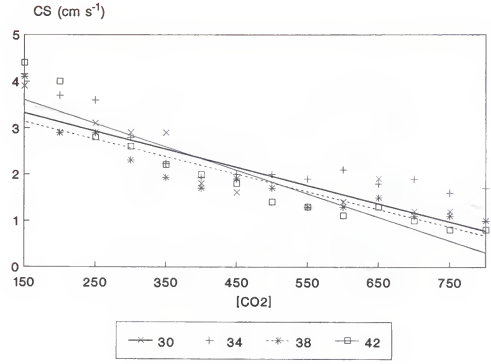

Figure 4-5. Stomatal conductance (cm s^{-1}) for 'Rotundifolia' holly after 21 days at root-zone temperatures of 30, 34, 38 and 42 C. Each value is the mean of four replicate plants. The relationship between C_s and CO_2 at each root-zone temperature were described as follows: 30 C:(R^2=0.87, C_s=4.00-0.0043(CO_2)), 34 C:(R^2=0.78, C_s=4.12-0.0035(CO_2)), 38 C:(R^2=0.79, C_s=3.93-0.0041(CO_2)) and 42 C:(R^2=0.80, C_s=6.33-0.0074(CO_2)).

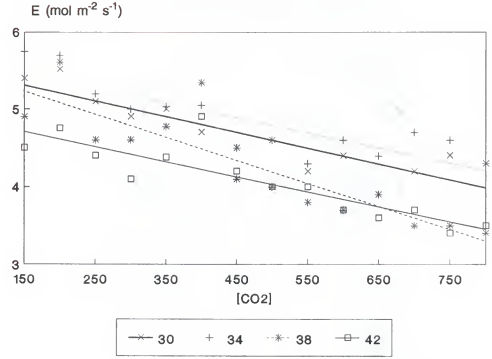

Figure 4-6. Transpiration values (mol m^{-2} s^{-1}) for 'Rotundifolia' holly after 21 days at root-zone temperatures of 30, 34, 38 and 42 C. Each value is the mean of four replicate plants. The relationship between E and CO_2 at each root-zone temperature was described by the following equations: 30 C:(R^2=0.79, E=5.61-0.002(CO_2)), 34 C:(R^2=0.73, E=5.76-0.002(CO_2)), 38 C:(R^2=0.76, E=5.66-0.003(CO_2)) and 42 C:(R^2=0.75, E=4.99-0.002(CO_2)).

In conclusion, different aspects of photosynthesis responded differently to increasing root-zone temperature. Decreased stomatal limitation at 34 C was related to increased CO_2 assimilation whereas no difference was shown between other root-zone temperatures. Photosynthetic pigment levels decreased while soluble protein levels increased with increasing root-zone temperatures. Cytokinins from roots are known to prevent the degradation of photosynthetic pigments and proteins. Decreased cytokinin levels were seen in apple trees in response to supraoptimal root-zone temperatures (Gur et al., 1972). Kuroyanagi and Paulsen (1988) demonstrated that high root-zone temperatures (35 C) in wheat increased the activities of protease and RNase enzymes and increased the loss of chlorophyll, protein and RNA from shoots. Since no differences were seen in fluorescence parameters, indicating that electron transport systems were not damaged, differences in assimilation may be due to decreased interception of quanta caused by decreased pigment levels. Further research regarding RuBisCO activation state and total RuBisCO levels would be necessary to describe differences in RuBisCO activity seen in this study.

CHAPTER 5

SUMMARY AND CONCLUSIONS

Experiments were conducted to determine the effects of supraoptimal root-zone temperatures on: 1) fixation and distribution of ^{14}C photosynthates, 2) root respiration and 3) physiological and biochemical responses related to photosynthetic processes in Ilex crenata Thunb. 'Rotundifolia'. All plants were grown in controlled environment walk-in growth rooms. Root-zone temperatures were maintained at 30, 34, 38 and 42 C for six hours daily over a 21 day period for all experiments.

In the first experiment using split-root 'Rotundifolia' holly plants, 38 C was the upper threshold temperature for a number of growth and physiological parameters. Higher root-zone temperatures had no effect on photosynthetic rates or root:shoot ratios but, altered photosynthate partitioning to different stem and root sinks. Although no differences were found for total ^{14}C partitioned to the roots, partitioning of ^{14}C into insoluble and soluble fractions and the magnitude of root respiration and leakage was influenced by treatment. Heating half of a root system to 38 C increased the amount of ^{14}C respired from the heated side and increased the amount of total CO_2 respired from the non-heated (30 C) half. Exposure of both root halves to 42 C resulted in substantial loss of ^{14}C through leakage into the media, presumably due to membrane damage.

Respiration of excised roots as influenced by root-zone growth temperature and buffer solution temperature was measured in the presence and absence of SHAM and KCN. Respiration rates for excised roots grown at 30, 34, 38 and 42 C decreased linearly with increasing root-zone temperature when the buffer solution was maintained at 25 C. When the

buffer solution temperature was the same as the root-growth temperature, growth temperature did not affect respiration rate. Increased respiration rates at 38 and 42 C when measured at the same buffer solution temperature, compared to rates from roots in buffer solution at 25 C, were ascribed to changes in activation energy of respiratory enzymes. Lack of differences in respiration rate when respiration was measured at the same temperature as the growth temperature was in agreement with the results of the first experiment. Respiration in roots from plants grown at a root-zone temperature of 30 C was maximum at a buffer solution temperature of 34 C and reached a minimum at 46 C. Above 46 C, a stimulation of O_2 consumption occurred which was presumed to be extra-mitochondrial in nature. The lethal temperature for 'Rotundifolia' holly roots was predicted to be 48±1.5 C for a 30 minute exposure. A 10 minute exposure to 46 C decreased respiration rates and damaged CN-resistant pathway activity. In the first experiment, a three-week exposure to 42 C caused sufficient membrane damage to increase root leakage of labelled assimilates.

In the final experiment, increased CO_2 assimilation at a root-zone temperature of 34 C was attributed to decreased stomatal limitations. CO_2 assimilation at 38 and 42 C was not different from that observed at 30 C. Chlorophyll *a* fluorescence parameters were not influenced by root-zone temperature, indicating that photosynthetic electron transport was not affected. Differences in the linear phase of the relationship between CO_2 assimilation and c_i indicated that activity was increased at 30 and 34 C compared to 38 and 42 C. Pigment levels decreased with increasing root-zone temperature. Further studies regarding RuBisCO activation state and protein levels are required before the response of 'Rotundifolia' holly can be thoroughly explained.

Lack of response of CO_2 assimilation to root-zone temperature treatments up to 42 C for 21 days is in contrast to the results of Foster (1986), who found decreased CO_2 assimilation after seven days at high root-zone temperatures. There was no evidence in either experiment of stomatal inhibition of photosynthetic CO_2 fixation due to supraoptimal root-zone temperature.

Differences in photosynthetic metabolism were found, but lack of differences in CO_2 assimilation rate at increased root-zone temperatures indicated that plants altered metabolism to acclimate.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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